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A detailed investigation to study the pattern of the interplay of Cyclic AMP Receptor Protein (CRP) of *E. coli* with its different classes of promoters

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KEYWORDS

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Transcription activation

CAP

CRP

AR1

AR2

AR3

ABSTRACT

The activity of most of the promoters in *Escherichia coli*, involved in the metabolism of sugars other than glucose, is controlled by a CRP (cAMP receptor protein) or CAP (catabolite activator protein). CRP-dependent promoters are differentiated into various classes (Class I, Class II, and Class III) based on its cognate binding site's position on DNA. The promoters regulated by CAP are differentially regulated by this transcriptional factor and it is also imperative to mention that these promoters vary greatly in respect to the binding site of CAP to its cognate binding site, it has also been reported that either it overlaps with the binding site of RNA polymerase or it present upstream to it. In Class I CAP-dependent promoters, a particular CAP molecule makes protein-protein interaction for the start of transcription. In Class II CAP-dependent promoters, a particular CAP molecule makes multiple interactions for the start of transcription. At last, in Class III-CAP dependent promoters, more than one CAP molecule is involved and activation of transcription is done synergistically. It has also been documented that CAP shows a kind of biphasic behavior in some promoters. So, the main focus of this work is to find out whether this biphasic behavior is true for other *E. coli* promoters as well. Experiments have been performed to know more about this biphasic nature and the various patterns of interactions of catabolite activator protein (CAP) of *E. coli* with its different classes of promoters.

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1 Introduction

Gene regulation is important for all organisms and *E. coli* is the most suitable organism to study this gene regulation process. It has already been known to us that there are several transcription factors present that control the gene regulation and one of them is Cyclic AMP receptor protein (CRP) or Catabolite activator protein (CAP). The gene regulation of nearly 150 promoters is controlled by this DNA binding protein (de Crombrugghe et al. 1984; Busby and Ebright 1999; Lawson et al. 2004). As it is evident from its name, it is a cAMP (cyclic adenosine monophosphate) binding protein, which activates transcription only in the presence of this nucleotide. In the absence of this nucleotide, this transcriptional factor is inactive, but after binding of cAMP, the transcriptional factor undergoes a conformational change and activates transcription at various promoters specifically those which are involved in the metabolism of alternative carbohydrate sources like lactose, galactose, maltose, arabinose, etc. other than glucose (Lawson et al. 2004; Ebright 1993). Although CRP is a cAMP binding protein, this protein exhibits a clear biphasic dependence over the varying concentration of cAMP, and the cAMP level is more important rather than its bare existence.

The protein, *E. coli* CAP is homo-dimeric and its every subunit consists of 209 aa residues and each subunit has two domains. Each subunit is also consisting of an HTH motif responsible for DNA binding (Mckey and Steitz 1981). The binding of this transcriptional activator is fixed on the region of the promoter to activate or repress transcription. This site is a cognate 22 bp consensus sequence (5'-AAATGTGATCTAGATCACATTT – 3'), having a twofold sequence symmetry (Ebright et al. 1989; Ebright 1993). It has also been stated that the complex of this protein with DNA slightly bends the DNA that is about 90º in the crystalline state and about 80º to 180º in solution (Lawson et al. 2004).

To activate transcription, CAP needs to bind to its cognate site on the promoter DNA along with RNA polymerase (RNAP)[3]. The promoters, that are regulated by the CAP-cAMP complex are of various categories based on the appearance of CAP site present in the P/O region and also the pattern of interaction of CAP with RNAP. There are three different classes present, which are - Class I, Class II, and Class III. The binding site for CAP can be -41.5, -61.5, -71.5, -82.5, etc. In the case of Class I promoter the transcriptional activation involves interaction between the activating region 1 (AR1) of CAP and RNAP, whereas, in Class II, the mode of interaction is a little different and in this case the interaction mainly involves activating region 1 (AR1), activating region 2 (AR2) and activating region 3 (AR3) of CAP and RNAP. But, In the case of class III, two or more CAP molecules are involved and interaction with RNAP involves the mechanism of both Class I and Class II (Ebright 1993; Busby and Ebright 1997; Lawson et al. 2004).

In this paper, we have discussed briefly about CAP and its three different classes of promoters i.e., Class I, Class II, Class III promoters, and also we have done experiments to find out whether the biphasic character of cAMP is observed in all these classes of promoters or not.

1.1 What is CRP or CAP?

CRP or CAP is a global transcriptional protein that involves primarily in the regulation of transcription of promoters mainly involves in the metabolism of sugars other than glucose. As already mentioned, it has two subunits, and each subunit of the CAP has two domains, one N-terminal domain (amino acids 1-133), involved in cAMP binding, and a C-terminal domain (amino acids 139-209), involved in DNA binding (Tutar 2008). These domains are linked by a small hinge region consisting of amino acids 134-138 (Tutar 2008).

Energy conservation is crucial for all organisms and bacteria is also no exception to this rule and at certain metabolic conditions bacterial cells overexpress certain proteins where expressing other proteins at the basal level, as the presence of glucose in the medium inhibits the cAMP production and thus activation of CAP does not take place, but when glucose is exhausted in the medium, cAMP concentration increases and which in turn binds with CAP and thus CAP activates transcription of promoters (Kolb et al. 1993a; Sharma et al. 2009). Binding of this nucleotide results in a geometrical change in the protein from an inactive shape to an active shape. This active conformer can bind to its cognate sites on DNA and activates or repress transcription (Sharma et al. 2009; Saha et al. 2015). The binding of cAMP to the un-liganded CAP brings certain biochemical and biophysical changes in the protein and it has been observed that after cAMP binding (a) CAP becomes more sensitive to various proteases, (b) it also reduces the C178 (cysteine residue) accessibility and (c) also allows the intersubunit cross-linking at the C178 residue by a disulfide linkage, apart from other conformational changes as probed by various spectroscopic studies (Kolb et al. 1993a; Saha et al. 2015).

It has been already mentioned that CAP undergoes changes in shape upon cAMP binding, but since 2009, the exact molecular mechanism behind this conformational change remains elusive. Though several crystal structures of this protein were available, the crystal structure of un-liganded CAP or apo-CAP was not there. The apo-CAP (CAP without cAMP) structure was solved using X-ray crystallography technique and NMR spectroscopy technique in the year 2009 (Ryu et al. 1993; Sharma et al. 2009). Various structural changes have been observed between the structure of inactive form i.e. apo-CAP and active form i.e. ligand-bound CAP (cAMP-CAP) (Popovych et al. 2009). The changes are mentioned in Table 1.

Table 1 Changes within the inactive form of CAP (apo-CAP) and active form of CAP (cAMP-CAP).

Parts of CAP structure	Amino acids (Inactive form)	Amino acids (Active form)	Reference
D-Helix	135-152	139-152	Sharma et al. 2009
C-Helix	110-130	110-136	Saha et al. 2015
F-Helix	Buried inside DNA binding domain	Comes outside on the surface	Sharma et al. 2009
Hinge	130-134	136-139	Sharma et al. 2009

As already mentioned, in presence of cAMP, CAP becomes more susceptible towards various proteases and this particular change in protease sensitivity between the active and inactive form of CAP are due to these structural changes. Protease like chymotrypsin cleaves CAP at F136. The un-liganded form of CAP is resistant to protease because, in the inactive form of CAP, the F136 present within the rigid structure of D-helix and cAMP binding brings the F136 residue in the hinge region. For this reason, the active form of CAP becomes more susceptible to proteases (Garges and Adhya 1985; Sharma et al. 2009).

The most interesting character of CAP is its biphasic dependence over the varying concentration of cAMP. Previously it was thought that only 2 molecules of cAMP bind to CAP to its N terminal domain, but later on it was discovered that instead of 2, 4 molecules of cAMP can bind the protein at a higher cAMP concentration. Out of these 4 molecules, 2 bind to the NTD, and 2 bind to the CTD. It has also been noted that cAMP bind at the NTD in anti-conformation and these are the high-affinity sites, whereas the CTD binding sites are low-affinity sites, and cAMP bind in this domain in the syn-conformation (Passner and Steitz 1997; Mukhopadhyay et al. 1999). Actually, without cAMP the CAP is inactive and at a low level it becomes active, but again at higher concentration, it behaves like the un-liganded CAP, thus inactive. Biophysical experiments also proved that this structure is very much similar to un-liganded CAP (Heyduk and Lee 1989). This biphasic behavior of CAP has been proved by several experiments. When the sensitivity of CAP to proteases has been observed for various concentrations of cAMP then it was found that without cAMP the CAP is resistant to proteases, at low cAMP concentration the CAP is sensitive to proteases and at high concentration of cAMP, the CAP is again becoming resistant to proteases (Mukhopadhyay et al. 1999). Also, it has been found that the binding of cAMP decreases the accessibility of cysteine residues at 178 positions (C-178)[16]. The same kind of biphasic behavior can also be observed in the case of transcriptional activation of certain promoters as well (Mukhopadhyay et al. 1999).

1.2 Transcriptional regulation by cap

As already mentioned, CRP-dependent promoters are divided into three types- Class I CRP-dependent promoters, Class II CRP-dependent promoters, and Class III CRP-dependent promoters.

1.2.1 Class I CRP-dependent promoters

In Class I CRP-dependent promoters, the CRP binding site on double helix DNA is located in the upstream position of the RNA polymerase (RNAP) binding site (Ebright 1993; Lawson et al. 2004). An example of this promoter is *lac* promoter having CRP binding site at -61.5 position. *In vivo*, the other CRP binding sites in this promoter can be -72, -82, -92 positions (Ebright 1993; Busby and Ebright 1994, 1999; Lawson et al. 2004).

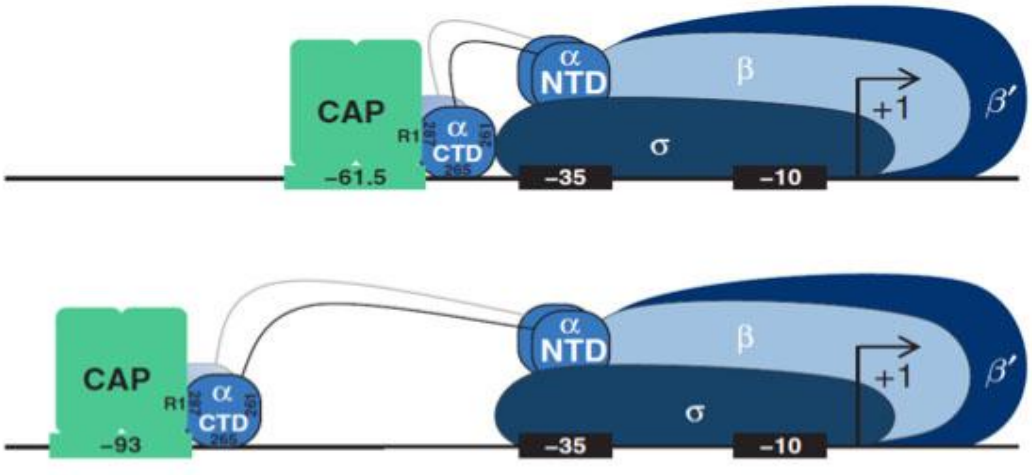

Figure 1 Transcription activation by Class I CAP-dependent promoter (adapted from Georis 2013)

Here, amino acid residues '156-164' of the downstream subunit of CRP denotes as 'activating region 1 (AR1)' and this region is mainly responsible for transcription activation in this promoter (Bell et al. 1990; Niu et al. 1994; Zhou et al. 1993). The AR1 interacts with the '287 determinant' of αCTD part of RNAP. This interaction allows binding of αCTD to DNA with its '265 determinant'. The αCTD binds with the σ^{70} subunit of RNAP with its '261 determinant'. Thus with these three determinants, transcription activation takes place and any mutation in these three determinants can cause defective transcription (Igarashi and Ishihama 1991; Murakami et al. 1996; Savery et al. 1998, 2002; Lawson et al. 2004).

There is a protein-protein interaction occurring in this Class I CRP-dependent transcription activation (Ebright 1993; Busby and Ebright 1999; Lawson et al. 2004) (Figure 1). Interaction occurring between CAP and RNAP makes RNAP-promoter DNA interaction stronger. When there is an increase in affinity, the binding constant, K_B also increases which forms the closed complex (RNAP-promoter) and thus initiation of transcription is also increased (Malan et al. 1984; Kolb et al. 1993b; Heyduk et al. 1993; Law et al. 1999).

1.2.2 Class II CRP-dependent promoters

In this promoter, the CRP binds on a region on double-helix DNA overlapping the binding site of RNA polymerase (RNAP). In vivo, -41.5 position on DNA strand denotes the site where CRP binds (Lawson et al. 2004). *GalP1* promoter is one of the best-known examples of Class II CRP-dependent promoters (Busby and Ebright 1997; Lawson et al. 2004).

It has been noted that all the three activating regions 'AR1', 'AR2', and 'AR3' are involved in this transcription activation.

AR1- This region is similar to Class I CRP-dependent promoter but the only difference is that in Class II CRP-dependent promoter AR1 is present in the upstream subunit of CRP (West el al. 1993; Zhou et al. 1994a, 1994b). The AR1 interacts with the '287 determinant' of one αCTD (Niu et al. 1996).

AR2- This region consists of residues "His19, His21, Glu96, and Lys101" and is present in the downstream subunit of CRP. The charge of AR2 is positive and it interacts with residues 162-165 of one αNTD (Niu et al. 1996; Rhodius et al. 1997).

AR3- This region is made by replacing Lys52 with an amino acid residue, which is mainly neutral or negatively charged. Residues 52-58 denote AR3 (Bell et al. 1990; Williams et al. 1991; West et al. 1993; Niu et al. 1996). AR3 is workable only in the downstream subunit of CAP (Williams et al. 1996). This AR3 interacts with the σ^{70} (residues 590-600) subunit of RNAP (Busby and Ebright 1997, 1999). Thus, there are three protein-protein interactions observed in this promoter (Figure 2).

1.2.3 Class III CRP-dependent promoters

In Class III CRP-dependent promoters, a single CRP dimer is not involved but rather two or more are involved. These CAP dimers collectively activate transcription in a few promoters. Here the distance between CAP binding sites on DNA is varied and the distance between CAP binding sites and RNAP binding sites are also different. The transcription activation in this class of promoters is really simple as it only involves the combination of Class I and Class II promoters (Busby and Ebright 1999).

If for example one CAP dimer is present at the -62 position and another CAP dimer is present at the -82 or -92 position then both can activate transcription synergistically through the Class I mechanism (Busby and Ebright 1999; Law et al. 1999). Similarly, if one CAP dimer is present at -62, -72, -82, or -92 position and another CAP dimer is present at -41.5 position then also both can activate transcription synergistically through the Class I mechanism is the upstream CAP subunit and Class II mechanism in the downstream subunit (Murakami et al. 1997; Busby and Ebright 1999, 1994) (Figure 3). In Class III CRP-dependent promoters both copies of αCTD are involved. In Class III CRP-dependent transcription activation both the CRP dimers make free contact with various surfaces of RNAP as well as no direct contact is required between CRP dimers (Busby and Ebright 1999).

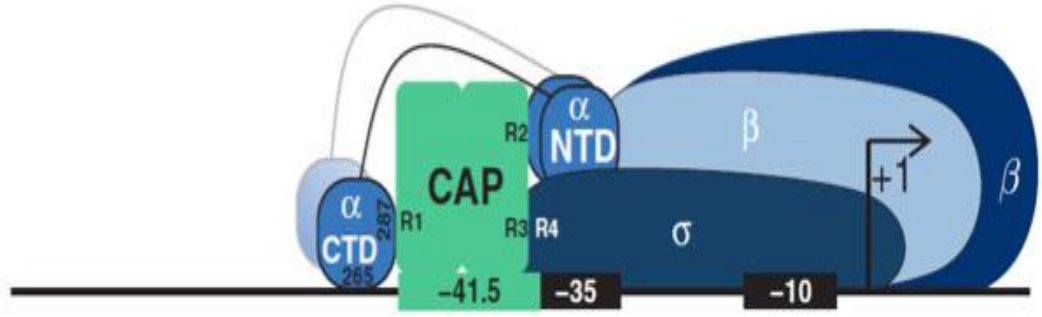

Figure 2 Transcription activation by Class II CAP-dependent promoter (adapted from Georis 2013)

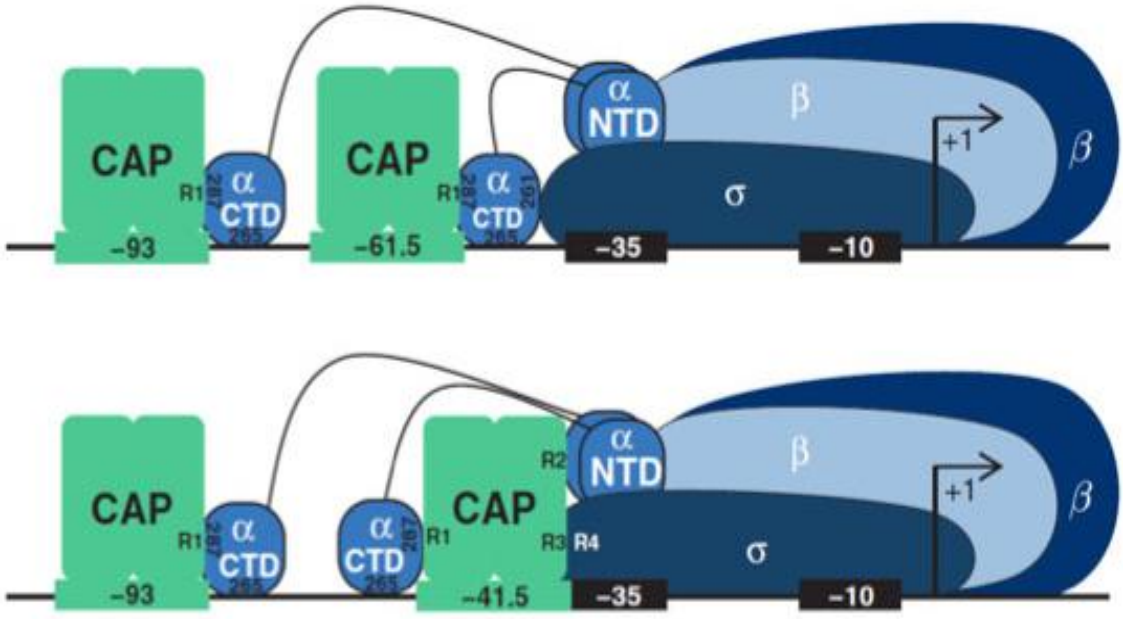

Figure 3 Transcription activation by Class III CAP-dependent promoter (adapted from Georis 2013)

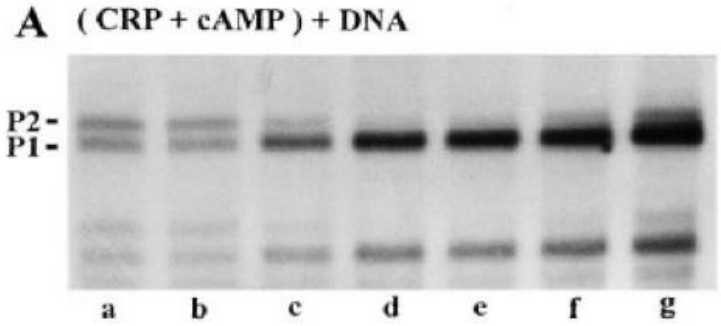

Figure 4 The effect of cAMP on *In vitro* transcription from *gal* promoters. Run-off transcripts from P1 and P2 promoters are indicated. cAMP concentrations used were 0, 0.2, 2, 20, 100, 200 and 400 μM (lanes a to g). At low level of cAMP, the transcript from P2 is less, but further addition of cAMP tends to reverse this effect.

2 Biphasic Behaviour of Cap

As it has been stated earlier that there is a clear biphasic behavior that CAP follows upon binding of cyclic AMP or cAMP. This biphasic behavior is very much evident and reported in Class II CRP-dependent promoters and more specifically in *gal* promoters (Mukhopadhyay et al. 1999). Our main objective of this work is to find out whether this biphasic character is observed only in *gal* promoters or is a general phenomenon for other promoters as well? To answer this question, *in vitro* transcription experiments and docking were done with other promoters of *E.coli*.

In the case of *gal* promoter, which is a Class II CAP-dependent promoter, the operon consists of two promoters P1 and P2 which overlap each other. It has been reported that when cAMP is absent, both the promoters produce transcripts at a basal level, there is no activation from either of the promoter but the addition of cAMP activates and initiate transcription, moreover, in the presence of a low level of cAMP, only P1 is activated and whereas the transcription from the other promoter i.e., P2 is repressed and thus transcripts are produced only from P1.So, at a low level of cAMP, gal P1 is activated and gal P2 is repressed but this pattern is only observed up to a certain concentration of cAMP (Figure 4). Moreover, further addition of cyclic AMP tends to alter this effect and further addition of cAMP tends to activate P2 whereas inhibits P1. This shows that *gal* promoter shows biphasic characteristics for the concentration of cAMP (Mukhopadhyay et al. 1999).

Table 2 Sequence of Primers used to generate a template for *In-vitro* Transcription

Name of the Primer	Sequence (5'-3')	Promoter region amplified
LacPF	CGCCCATATGGTTGGCCGATTCATTAATGC	*Lac*
LacPR	TTAGGGATCCATTACGCCAGCTGGCGAAAG	*Lac*
ABS 102	ATCGCCGCGCGTCACCATCG	*Cya*
ABS 103	TGATTCCGCCAACATCAACG	*Cya*

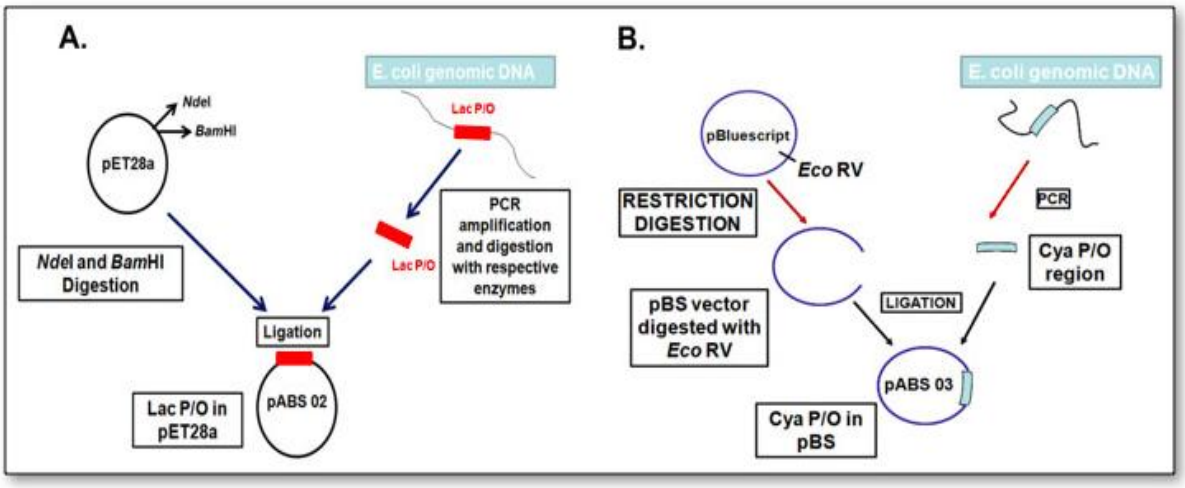

Figure 5 A schematic representation of cloning of lac (A) and cya (B) promoter region of *E. coli* into pET28a and pBluescript vector respectively.

3 Materials and Method

3.1 Preparation of DNA fragments for In vitro transcription experiment with the various promoter and operator regions of E. coli

For *in vitro* transcription experiment, 300 bp *lac* promoter operator region was amplified and cloned with suitable primers LacPF and LacPR (Table 2) as reported earlier (Saha et al. 2015). The –370 to +120 region of *E. coli cya* promoter was also amplified by PCR from *E. coli* genomic DNA employing suitable oligos (ABS 102 and ABS 103) and cloned into pBluescript vector at *Eco* RV site to obtain plasmid pABS 03. A schematic representation of cloning of lac and cya promoter regions is shown in Figure 5.

3.2 In vitro transcription

The IVT "*In vitro* transcription" reactions were done in 20 µl reaction volume with Lac P/O and Cya P/O region as described earlier The product was estimated by running a denaturing gel, and finally, the RNA product was estimated with a scanner system.

To investigate the biphasic cAMP dependence in other promoters, *in vitro* transcriptions reactions of CAP were also done in the presence of the varying amount of cAMP for both *lac* and *cya P/O* regions of *E. coli* as described above. The final concentrations of cAMP were 0, 0.3, 0.75, 2, 20, 50, 200, 400, 800 and 1000 µM respectively in case of *lac P/O* and 0, 50, 100, 200, 400, 800, 1000 µM respectively in case of *cya P/O*.

3.3 Docking of Lac and Gal promoters

The DNA sequences of the *Galactose* & *Lactose* promoter of *E. coli* were collected from the article (Kolb et al. 1983). Two 3D models (*Lac*&*Gal* promoter) were built with Avogadro: Molecular Editor and Visualization software (https://avogadro.cc/). After the 3D model was prepared then we have edited the structure by Text-Pad, we have changed the DNA two letters code to a three letters code for docking as the HADDOCK server accepts DNA three letters code.

The *E.coli* CAP-cAMP complex crystal structure (PDB:2GZW) was downloaded from online resources (Protein Data Bank). The protein has 4 chains (A,B,C,D) so, we have edited the structure in Text-Pad and cleave the chains Both C and D, and the final which we have made has two chains A and B.

The DNA-Protein Docking was carried out with HADDOCK 2.4 server (https://wenmr.science.uu.nl/haddock2.4/) (van Zundert et al. 2016). In the HADDOCK server two input molecule files were required that is DNA and Protein. So, we put the protein file which was the CRP-cAMP complex file in input molecule no.1, and the

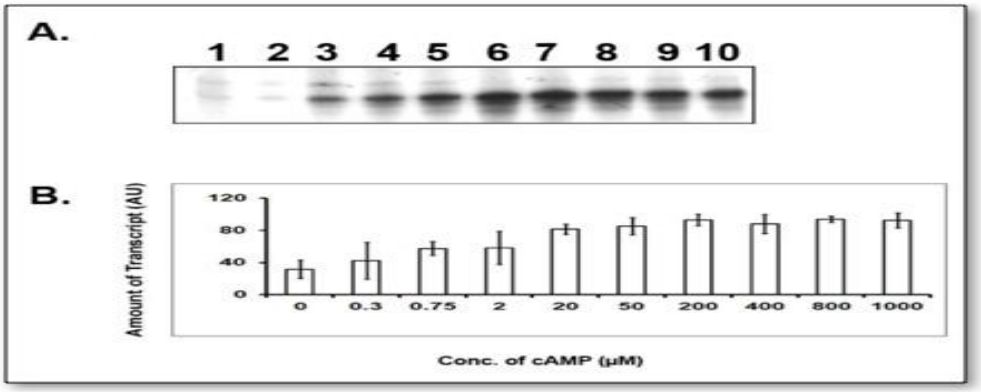

Figure 6 In vitro transcription of *lac* P/O with different concentrations of cAMP (A. CAP was preincubated with the indicated amount of cAMP on ice for 5 minutes, followed by addition to the reaction mixture containing 5 nM of template and 50 nM of RNA polymerase, followed by further incubation at 37^0C for 20 minutes. Transcription was initiated by the addition of the NTP mix, as described under Methods. After another 20 minutes, the reaction was stopped and the products were analyzed on a 10% polyacrylamide-7 M urea gel. Lanes 1-10, 0, 0.3, 0.75, 2, 20, 50, 200, 400, 800 and 1000 µM cAMP. B. Histogram of the above, showing the amounts of transcript produced as a function of cAMP concentration).

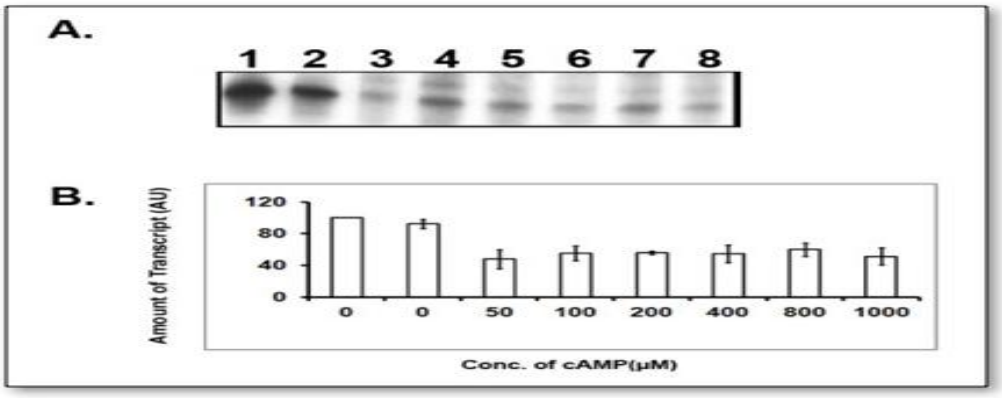

Figure 7 In vitro transcription of cya P/O with different concentrations of cAMP (A. The run off transcripts from *cya*p/o regions were analyzed on a 10% polyacrylamide-7 M urea gel. Lane 1, promoter alone; lanes 2-8, 100 nM CRP with 0, 50, 100, 200, 400, 800 and 1000 µM of cAMP. B. Histogram of the above, showing the amounts of transcript produced as a function of cAMP concentration)

DNA file which was the *Galactose* promoter file in input molecule no.2. The same process was followed in the case of Lactose promoters. After that, the parameter was given manually like the active residues and the chains which are involved in the interaction. At last, we selected the option in which the passive residues will be automatically taken.

After Docking the DNAproDB server (https://dnaprodb.usc.edu/index.html) was used to visualize the data (Sagendorf et al. 2020).

4 Results

4.1 In vitro transcription of CAP with lac and cya promoter regions at varying concentrations of cAMP

It has been suggested that two different shapes (conformers) of CAP present at low and high cAMP concentrations and these conformers behave differently regarding transcriptional regulation of *E. coli* gal promoters. To examine the probable function of these two conformers in the modulation of transcription in other promoters, we have done *in vitro* transcription experiments of CAP with *Lac* and *Cya* promoter/operator regions at a varying concentration of cAMP (Figure 6 and Figure 7).

In the case of the *lac* promoter/ operator region, the intensity of the promoter-specific transcript increased monotonously with varying concentrations of cAMP. Therefore, in the case of the *lac* promoter, clear monophasic transcription regulation by CRP with relation to cAMP concentrations is observed (Figure 6).

For *cya* promoter, the intensity of the promoter-specific transcript decreased monotonously with varying concentrations of cAMP (Figure 7). It has been reported earlier that transcription of the *E. coli ac* (adenylate cyclase) gene (i.e. *cya*) is regulated by the CAP-

Table 3 Docking parameter of CAP-cAMP with *gal* promoter and CAP-cAMP with *lac* promoter complex

Parameters	CAP-cAMP with *lac* promoter	CAP-cAMP with *gal* promoter
HADDOCK score	-92.1 +/- 1.7	-95.3 +/- 4.0
Size of the cluster	14	20
RMSD value (Lowest-energy structure)	31.3 +/- 0.1	29.9 +/- 0.4
V - W energy	-47.1 +/- 3.6	-47.9 +/- 4.0
Electrostatic energy	-483.1 +/- 18.0	-518.3 +/- 13.8
Desolvation energy	23.9 +/- 0.9	24.8 +/- 1.0
Restraints violation energy	277.0 +/- 48.4	313.9 +/- 21.8
Buried Surface Area	1783.6 +/- 89.2	1739.3 +/- 85.2
Z-Score	-1.3	-2.4

Table 4 Residues involved in interaction validated using DNAproDB web-based visualizing tool

Name	Interaction residues
CRP-cAMP with *lac* promoter	R180, E181, T182, G184, R185, V139, R385, T382, S179*
CRP-cAMP with *gal* promoter	R180, E181, T182, R185, V139, R385, S179*

Table 5 The docked complex structures are confirmed using DNA proDB (web-based) visualization tool (For CRP-cAMP with *lac* promoter)

DNA Entity ID	Pro. Chain ID	Pro. Chain Segments	Nuc-Res Interactions	Weak Nuc-Res Interactions	Total BASA [Å²]	Total H-bonds	Total vdW	Hydrophobicity Score (SAP)	Secondary Structure Composition
A1@A2	B	B1, B2	41	7	1010.126	20	98	-1.667	helix

Table 6 The docked complex structures are confirmed using DNA proDB(web-based) visualization tool (For CRP-cAMP with *gal* promoter)

DNA Entity ID	Pro. Chain ID	Pro. Chain Segments	Nuc-Res Interactions	Weak Nuc-Res Interactions	Total BASA [Å²]	Total H-bonds	Total vdW	Hydrophobicity Score (SAP)	Secondary Structure Composition
A1@A2	B	B1, B2	40	5	904.017	23	80	-1.815	helix

BASA= buried solvent accessible surface-area; vdW= V - W interaction; SAP= spatial aggregation propensity algorithm

cAMP complex in a negative manner. Therefore, it is clear from these two observations that CAP showed a monophasic behavior upon varying concentrations of cAMP, for the inducible promoter *lac* as well as the repressible promoter *cya* or in other words CAP does not show biphasic behavior upon varying cAMP concentrations universally in all promoters.

4.2 Docking of Class I and Class II Promoters with CRP

About 10 clusters were obtained after the docking and among them, the cluster having the lowest Z value and RMSD value were taken. The Z value, RMSD value, and restraints value of *gal* promoter is much less than that of the *lac* promoter. The best complex is also found out by using SASA (solvent accessible surface area) parameters for each molecule and also for the complex. All the parameters were mentioned in Table 3. The desolvation energy of the *gal* promoter is also more. CAP-cAMP complex interacting with *gal* promoter provided the most suitable complex structure with (-95.3 +/-4.0) HADDOCK score. It has also been observed that another parameter, the RMSD value from the lowest-energy structure is less in the case of *gal* promoter that's why *gal* promoter indicates a good stable structure.

Table 4 shows the residues of CAP-cAMP involved in the interaction with both the promoters. The interactions between these residues of CAP-cAMP with *lac* promoter (Figure 8) and with *gal* promoter (Figure 9) were also observed. The docked complex structures of *lac* and *gal* promoters are mentioned in Table 5 and 6. Lastly, through docking the structure of *lac* promoter with CAP-cAMP (Figure 10) and *gal* promoter with CAP-cAMP complex (Figure 11) were obtained.

It has been observed through this experiment that CRP-cAMP with *lac* promoter complex has two more interactions (G184, S382) other than CAP-cAMP with *gal* promoter. Moreover, it is imperative to note that *S179 residue interacts with the DNA loop (Table 4).

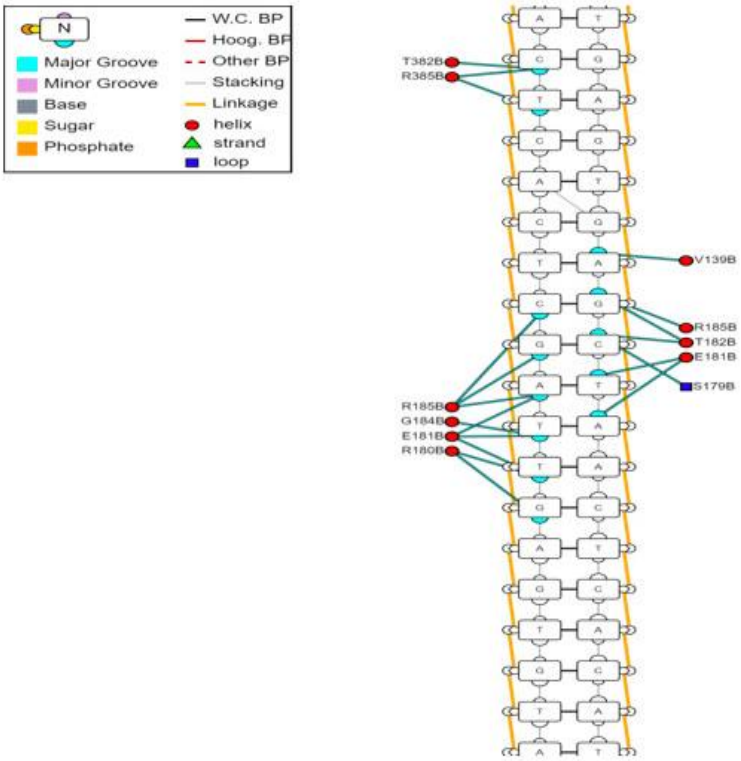

Figure 8 Interaction between CAP-cAMP and *lac* promoter

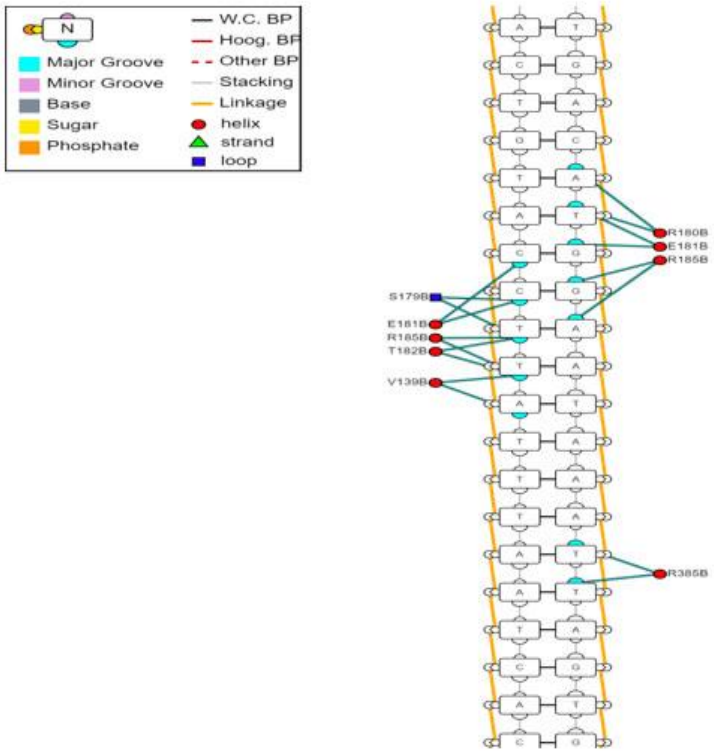

Figure 9 Interaction between CAP-cAMP and *gal* promoter

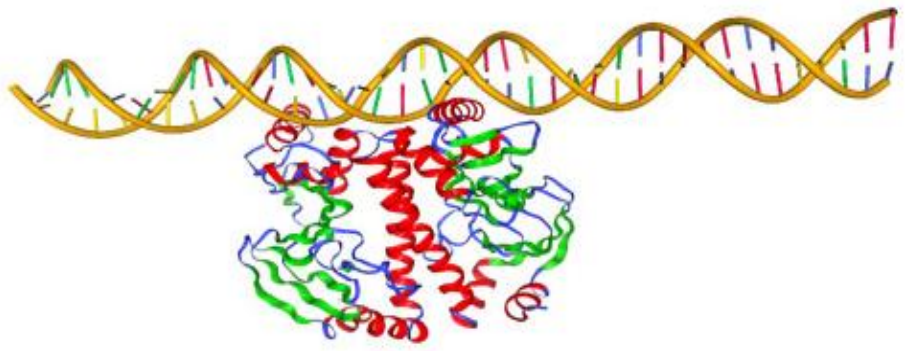

Figure 10 Docking complex of *lac* promoter and CAP-cAMP complex (The CAP-cAMP complex (red, green, blue) binds with the CAP binding site on the *lac* promoter DNA - yellow).

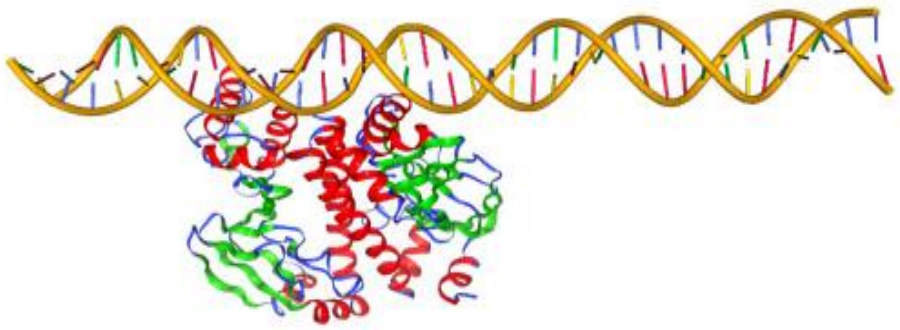

Figure 11 Docking complex of *gal* promoter and CAP-cAMP complex (The CAP-cAMP complex (red, green, blue) binds with the CAP binding site on the *gal* promoter DNA - yellow).

5 Discussions

So, it is clear from the result that the biphasic dependence of CAP with varying concentrations of cAMP might not be a general phenomenon. Though, the exact reason behind this difference was not clearly known but an assumption was made regarding this difference. It has been reported earlier that the residue Lys52 of CAP is important for transcriptional activation especially in Class II promoters and mutation of this residue affect Class II CAP-dependent transcription. cAMP binding site has a proximity towards Lys52. In the syn-cAMP complex, there is a conformational change of that part of the cAMP binding site which contains Lys52. If this CAP-cAMP complex bound to DNA interacts with any other protein, then the affinity of CAP for syn-cAMP will increase, and thus the conformation of CAP protein binding syn-cAMP will also get stabilized (Passner and Steitz 1997).

Abbreviations

cAMP- cyclic AMP; RNAP- RNA polymerase; αCTD- RNAP α subunit C-terminal domain; αNTD- RNAP α subunit N-terminal domain; AR1- activating region 1; AR2- activating region 2; AR3- activating region 3.

Conflict of Interest

There is no conflict of interest.

Author's Contribution

AS: conceptualization, investigation, writing original draft, supervision; SC: Investigation, writing, original draft, editing; SS: Computational investigation; RM: writing, editing; RM: conceptualization, supervision.

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