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Catabolite activator protein (CAP): DNA binding and transcription activation

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Abstract

Recent structures of *Escherichia coli* catabolite activator protein (CAP) in complex with DNA, and in complex with RNA polymerase α subunit C-terminal domain (α CTD) and DNA, have yielded insights into how CAP binds DNA and activates transcription. Comparison of multiple structures of CAP-DNA complexes has revealed contributions of direct readout and indirect readout to DNA binding by CAP. The structure of the CAP- α CTD-DNA complex has provided the first structural description of interactions between a transcription activator and its functional target within the general transcription machinery. Using the structure of the CAP- α CTD-DNA complex, the structure of an RNAP-DNA complex, and restraints from biophysical, biochemical, and genetic experiments, it has been possible to construct detailed three-dimensional models of intact Class I and Class II transcription activation complexes.

Keywords

catabolite activator protein (CAP); cAMP receptor protein (CRP); RNA polymerase; σ^{70} ; promoter; DNA binding; DNA bending; transcription activation

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Introduction

The *Escherichia coli* catabolite activator protein (CAP; also known as the cAMP receptor protein, CRP) activates transcription at more than one hundred promoters. CAP functions by binding, in the presence of the allosteric effector cAMP, to specific DNA sites in or near target promoters and enhancing the ability of RNA polymerase holoenzyme (RNAP) to bind and initiate transcription (reviewed in [1]). Transcription activation by CAP is a classic model system for structural and mechanistic studies of transcription activation. Thus, CAP was the first transcription activator to have been purified, was the first transcription activator to have its three-dimensional structure determined, and has been the subject of extensive biophysical, biochemical, and genetic investigations. Transcription activation by CAP at the simplest CAP-dependent promoters requires only three macromolecular components--CAP, RNAP, and promoter DNA--and requires only one DNA site for CAP [1]. Transcription activation by CAP at such promoters is simpler than most examples of transcription activation in bacteria (which require more numerous macromolecular components and/or DNA sites [2]), and very substantially simpler than examples of transcription activation in eukaryotes (which require tens of macromolecular components and DNA sites [3]). Accordingly, it has been possible to develop structural and mechanistic descriptions of transcription activation by CAP at such promoters that are more nearly complete than descriptions of any other examples of transcription activation.

The immediate scope of this article is to review results of recent structural and functional studies addressing the physical basis of DNA binding by CAP and the mechanism of transcription activation by CAP. Readers interested in cAMP binding by CAP and the cAMP-mediated allosteric transition in CAP are referred to recent articles reporting structures of the cAMP-liganded state of CAP [4,5] and the unliganded state of the CAP homolog CooA [6], recent articles describing effects of cAMP binding on the structure and dynamics of CAP in solution [7,8], and a recent review article [9].

DNA binding by CAP

CAP is a dimer of two identical subunits, each of which is 209 residues in length and contains a helix-turn-helix DNA-binding motif [10]. CAP interacts with a 22 bp two-fold-symmetric DNA site, 5'-AATGTGATCTAGATCACATTT-3' [11]. The CAP-DNA complex is two-fold symmetric: one subunit of CAP interacts with one half of the DNA site; the other subunit of CAP interacts with the other half of the DNA site (Fig 1 [12,13,14]). The initial structures of CAP-DNA complexes revealed two distinctive features [12,13,14]: First, CAP recognizes its DNA site through a combination of "direct readout" (DNA-sequence recognition mediated by direct hydrogen-bonded or van der Waals interactions with DNA base pairs) and "indirect readout" (DNA-sequence recognition mediated by sensing of DNA-sequence-dependent effects on DNA-phosphate position, DNA-phosphate solvation, or susceptibility to DNA deformation). Second, CAP bends DNA by $\sim 80^\circ$, wrapping DNA toward and around the sides of the CAP dimer. DNA bending is localized to two phased kinks in each DNA half-site: a "primary kink" of $\sim 40^\circ$ compressing the DNA major groove between positions 6 and 7, and a "secondary kink" of $\sim 9^\circ$ compressing the DNA minor groove between positions -1 and 2. Recent crystallographic and biophysical studies have shed further light on these issues.

Direct vs. indirect recognition

CAP exhibits strong sequence preferences at seven positions within each DNA half-site: positions 1, 2, and 4–8 (boxed positions in Fig 1b [11,15,16,17]). Sequence preferences at three positions within each DNA half-site--positions 5, 7, and 8--are accounted for by direct amino acid-base contacts (Fig 1b [12,13,14,18,19,20]). The guanidinium side chain of Arg180 forms hydrogen bonds with the guanine O⁶ and N7 atoms of the consensus base pair G:C at position

5; the carboxylate side chain of Glu181 forms a hydrogen bond with the cytosine N⁴ atom of the consensus base pair G:C at position 7; and the guanidinium side chain of Arg185 forms a hydrogen bond with thymine O⁴ atom of the consensus base pair A:T at position 8 (and, in some structures, also with the guanine O⁶ and/or N7 atoms of G:C at position 7). In contrast, sequence preferences at the remaining positions occur in the absence of amino acid-base contacts and thus must involve indirect readout (Fig 1b [12,13]).

DNA kinking and indirect recognition

The primary kink is located between positions 6 and 7 (Fig 1b). Positions 6 and 7 are part of a T:A/G:C base-pair step, a base-pair step that is associated with high roll angles and exceptional susceptibility to roll deformation [21]. It has been proposed that specificity for T:A at position 6 is a consequence of formation of the DNA kink between positions 6 and 7, and of effects of the T:A/G:C step on the geometry of DNA kinking, the energetics of DNA kinking, or both [13].

A recent study, involving a combination of biochemical and crystallographic approaches, showed that an amino-acid substitution in CAP that eliminates specificity for T:A at position 6 also eliminates formation of the primary kink (DNA structure in orange in Fig 2 [22*]). The results provide strong support for the proposed connection between specificity for T:A at position 6 and formation of the primary kink [22*].

A companion study showed that complexes of CAP with DNA sites having the consensus base pair T:A or the nonconsensus base pair C:G at position 6 exhibit similar overall DNA bend angles and local geometries of DNA kinking (DNA structures in yellow and red in Fig 2 [23*]). The results suggest that indirect readout in this system does not involve differences in the geometry of DNA kinking but, rather, solely differences in the energetics of DNA kinking [23*]. (However, it will be necessary to obtain structural data for complexes of CAP with DNA sites having G:C or A:T at position 6 in order to make a definitive statement.)

The results of both studies imply that the main determinant local DNA geometry in this system is protein-DNA interaction, and not DNA sequence [22*,23*].

DNA kinking and DNA smooth bending

In the first study described in the preceding section, protein-DNA interactions were shown to be similar in the structure of a CAP-DNA complex and in the structure of an [Asp181]CAP-DNA complex (wherein [Asp181]CAP is a CAP derivative having a Glu181→Asp181 substitution, a substitution that shortens the residue-181 side chain by one methylene group)--including even the hydrogen bond between the side-chain carboxylate of residue 181 and the cytidine N⁴ atom of G:C at position 7 [22*]. The overall DNA bend angles also were shown to be similar (DNA structures in orange and red in Fig 2 [22*]). However, the local DNA geometries at the primary-kink sites were shown to be radically different, with the CAP-DNA complex exhibiting a kink, and the [Asp181]CAP-DNA complex exhibiting a smooth bend (DNA structures in white and orange in Fig. 2 [22*]). The results indicate that a given overall DNA bend angle can be achieved through very different local DNA-helical parameters at the primary-kink site. The results further indicate that, in this case, the main determinant of local DNA-helical parameters at the primary-kink site is protein-DNA interaction, and not DNA sequence--with the protein in essence "bending DNA to its will."

We note, however, a complexity in the kinking vs. bending story. Two structures of wild-type CAP-DNA complexes solved in space group *P*3₁21 also exhibit radically different local DNA-helical parameters at the primary kink site: a kink in one structure [23*], and a smooth bend in the other structure [24]. The DNA fragments in the two structures differ in multiple respects,

including lengths (38 vs. 46 base pairs), sequences (consensus vs. nonconsensus at positions 1, 9, and 10 of each half-site) and positions of single-phosphate gaps (top strand vs. bottom strand at position 10 of each half-site). Therefore, in this case, it is not possible to deduce the basis for the difference in local DNA-helical parameters at the primary kink site.

DNA bending in solution

Nanosecond time-resolved fluorescence resonance energy transfer measurements between a probe incorporated in CAP and a complementary probe incorporated at each of a series of sites in the DNA indicate that the mean DNA bend angle in the CAP-DNA complex in solution is $77(\pm 3)^\circ$ degrees--a value consistent with the mean DNA bend angle observed in crystal structures, $80(\pm 12)^\circ$ [25*]. Lifetime-distribution analysis indicates that the distribution of DNA bend angles is relatively narrow, with <10% of DNA bend angles exceeding 100° [25*]. Millisecond time-resolved luminescence resonance energy transfer experiments provide independent evidence that the upper limit of the distribution of DNA bend angles is $\sim 100^\circ$ [25*].

What factors are responsible for DNA bending by CAP?

One factor is the DNA sequence of the consensus DNA site. Three studies find that the consensus DNA site for CAP contains an intrinsic bend in the absence of CAP [26,27,28]. Estimates of the DNA bend angle in the absence of CAP vary. An estimate of 15° was obtained in cyclization assays employing tandem arrays of DNA sites [26]. A much higher estimate of 52° was obtained in electrophoretic mobility shift phasing assays [28]. Millisecond time-resolved luminescence resonance energy transfer experiments indicate that the upper limit of the DNA bend angle in the absence of CAP is $40\text{--}50^\circ$ [27].

Another, likely more important, factor is formation of electrostatic interactions between positively charged residues on the sides of the CAP dimer (Lys22, Lys26, Lys44, Lys166, His199, and Lys201) and negatively charged DNA phosphates (positions -5 to -2) [12,15, 25*,27,28]. These electrostatic interactions are proposed to contribute to DNA bending in two ways: (i) by stabilizing the bent state through amino acid-phosphate interactions [12,15,25*, 27], and (ii) by destabilizing the unbent state through asymmetric phosphate neutralization [28]. Substitution of positively charged residues on the sides of the CAP dimer (Lys22, Lys26, Lys44, and Lys166) reduces the mean DNA bend angle in the CAP-DNA complex in solution by $\sim 5^\circ$ per residue per half-complex, as assessed in fluorescence resonance energy transfer and fluorescence anisotropy assays [27].

Transcription activation by CAP

Simple CAP-dependent promoters--*i.e.* promoters that require only CAP for transcription activation--can be grouped into two classes based on the position of the DNA site for CAP and the corresponding mechanism for transcription activation [1].

At Class I CAP-dependent promoters, the DNA site for CAP is located upstream of the core promoter. The best-characterized Class I CAP-dependent promoters are the *lac* promoter and the artificial promoter CC(-61.5), each of which has a DNA site for CAP centered at position -61.5. Transcription activation at Class I CAP-dependent promoters involves a single protein-protein interaction between CAP and RNA polymerase holoenzyme (RNAP; subunit composition $\alpha^I\alpha^II\beta\beta'\omega\sigma^{70}$ [29]), and proceeds through a simple "recruitment" mechanism, whereby CAP facilitates binding of RNAP to the promoter to yield the RNAP-promoter closed complex (Fig 3a [1]).

At Class II CAP-dependent promoters, the DNA site for CAP overlaps the core promoter, overlapping the core-promoter -35 element. The best-characterized Class II CAP-dependent promoters are the *galP1* promoter and the artificial promoter CC(-41.5), each of which has a DNA site for CAP centered at position -41.5 . Transcription activation at Class II CAP-dependent promoters involves three sets of protein-protein interactions between CAP and RNAP, and proceeds through both “recruitment” and “post-recruitment” mechanisms, whereby CAP both facilitates binding of RNAP to the promoter to yield the RNAP-promoter closed complex and facilitates isomerization of the RNAP-promoter closed complex to yield the RNAP-promoter open complex (Fig 3b [1]).

Transcription activation at Class I CAP-dependent promoters

At Class I CAP-dependent promoters, CAP activates transcription by binding to a DNA site located upstream of the core promoter and interacting with the RNAP α subunit C-terminal domain (α CTD), an 85 amino-acid residue independently folded domain that is flexibly tethered to the remainder of RNAP (Fig 3a [1,30]). Interaction of CAP with α CTD facilitates binding of α CTD--and, through it, the remainder of RNAP--to promoter DNA, and thereby stimulates transcription initiation.

Biochemical and genetic results indicate that the interaction between CAP and α CTD is mediated by “activating region 1” of the downstream subunit of CAP (AR1; blue in Fig 3a [31,32,33,34,35]) and the “287 determinant” of α CTD (yellow in Fig 3a [36]). Biochemical and genetic results further indicate that the interaction between α CTD and DNA is mediated by the “265 determinant” of α CTD (red in Fig 3a [36,37,38]) and the DNA minor groove [39]. At Class I CAP-dependent promoters where the DNA site for CAP is centered at position -61.5 , such as the *lac* and CC(-61.5) promoters, interaction between CAP and α CTD places α CTD adjacent to σ^{70} , and permits functional protein-protein interaction between α CTD and σ^{70} (Fig 3a [40**]). The interaction between α CTD and σ^{70} is mediated by the “261 determinant” of α CTD (white in Fig 3a [36,40**]) and residues 573–604 within the module of σ^{70} responsible for recognition of the promoter -35 element, σ^{70} region 4 (σ R4; pink in Fig 3a [40**]). RNAP contains two copies of α CTD: α CTD^I and α CTD^{II} (Fig 3a [29]). At Class I CAP-dependent promoters, one α CTD protomer--interchangeably α CTD^I or α CTD^{II}--interacts with CAP [1,39,41,42]. The other α CTD protomer interacts non-specifically with upstream DNA [1,39,43].

The recently determined crystal structure of a complex containing CAP, α CTD, and DNA has provided the first high-resolution structural description of the interaction between a transcription activator and its functional target within the general transcriptional machinery (Fig 4) [44**]. The interactions in the structure confirm, point-by-point, interactions predicted by biochemical and genetic results [44**]. Thus, CAP makes protein-protein interactions with α CTD, and α CTD makes protein-DNA interactions with the DNA minor groove adjacent to the DNA site for CAP. The interaction between CAP and α CTD is mediated by AR1 of CAP and the 287 determinant of α CTD (blue and yellow in Figs 3a, 4). The interaction between α CTD and DNA is mediated by the 265 determinant of α CTD (red in Figs 3a, 4) and the backbone and spine of hydration of the DNA minor groove adjacent to the DNA site for CAP. The 261 determinant of α CTD (white in Figs 3a, 4) is located on the face of α CTD opposite from CAP and is prominently exposed, consistent with availability to participate in interactions with σ R4.

Significantly, in the structure of the CAP- α CTD-DNA complex, there are no conformational changes in CAP and α CTD, and the interface between CAP and α CTD is small (six residues each of CAP and α CTD; 630 Å² of buried surface area [44**]). The small size of the interface, and the absence of conformational change in activator and target, are consistent with the

proposal that transcription activation at Class I CAP-dependent promoters involves a simple “recruitment” mechanism--*i.e.* simple “adhesive” interactions between activator and target that facilitate and/or stabilize interaction of the general transcription machinery with promoter DNA [1,30,44**,45,46]. (Activation by recruitment does not require conformational signalling within or through the target, does not require extensive, high-information-content interactions between activator and target, and entails modest net interaction energies between activator and target--interaction energies comparable to the magnitude of activation [45,46].) By joining the crystal structures of the CAP- α CTD-DNA complex [44**] and the σ R4-(–35 element) complex [47**]--simply superimposing DNA segments of the two structures onto a single, continuous DNA segment having a site for CAP, a site for α CTD, and a –35 element, spaced as at *lac* or CC(–61.5)--it has been possible to construct a provisional structural model for the CAP- α CTD- σ R4-DNA complex at a Class I CAP-dependent promoter such as at *lac* or CC(–61.5) [40**,48**]. The resulting model places the 261 determinant of α CTD adjacent to the 573–604 determinant of σ R4, permitting favorable electrostatic interaction between the determinants (which have, respectively, high net negative charge and high net positive charge) and permitting, with modest adjustment of side-chain torsion angles, direct contact between experimentally defined interacting residues. The fit between modelled and experimentally defined interactions is striking and can be further improved by moderate compression of the DNA major groove immediately upstream of the –35 element (*i.e.*, at positions –38 and –39 [40**,48**]). Figure 5a presents a structural model of the *intact, full* Class I CAP-RNAP-promoter complex at *lac*. The model was constructed in three steps: (i) joining the crystal structures of the CAP- α CTD-DNA complex [44**], the σ R4-(–35 element) complex [47**], and the RNAP-DNA complex [49**]--superimposing DNA segments of the three structures to generate a single, continuous DNA segment having a site for CAP, a site for α CTD, a –35 element, and a –10 element, spaced as at *lac*; (ii) refining local DNA-helix parameters immediately upstream of the –35 element (positions –36 to –41), using experimentally defined α CTD- σ R4 interactions [40**] and non-interpenetration as constraints, and using sequence-dependent DNA-deformation energies [50] as restraints; and (iii) modelling downstream DNA segments as in published models of the RNAP-promoter open complex [39,51**]. The model proposes moderate compression of the DNA major groove immediately upstream of the –35 element (~5° roll at the –38/–39 base-pair step). The model is consistent with all available experimental information and provides a structural framework for understanding Class I CAP-dependent transcription. An important feature of the model is that--due to consecutive phased DNA bends in the –35 element, the DNA immediately upstream of the –35 element, and the DNA site for CAP--essentially the entire upstream-promoter region between positions –40 and –100 is proposed to be in proximity to RNAP, and, in particular, the DNA minor grooves at positions –43, –53, –63, –73, –83, and –93 are proposed to be in proximity to α CTD^I and α CTD^{II} (Fig 5a). The proposed positions of upstream-promoter DNA minor grooves relative to α CTD^I and α CTD^{II} account for results indicating that the α CTD protomer not in contact with CAP can be crosslinked to the DNA minor groove at positions –73, –83, and –93 [39] and is available in principle to interact with a second activator in the –93 or –103 region [42, 52].

Transcription activation at Class II CAP-dependent promoters

At Class II CAP-dependent promoters, CAP binds at or near position –41.5 and makes three sets of protein-protein interactions with RNAP (Fig 3b [1]). “Activating region 1” of the upstream subunit of CAP (AR1; blue in Fig 3b [34,35,53,54]) interacts with the “287 determinant” of α CTD (yellow in Fig 3b [55]). “Activating region 2” of the downstream subunit of CAP (AR2; dark green in Fig 3b [56]) interacts with residues 162–165 within α NTD^I (orange in Fig 3B [1,41,56]). “Activating region 3” of the downstream subunit of CAP (AR3; olive green in Fig 3b [53,54,56,57,58,59]) interacts with residues 593–603 within the module of

σ^{70} responsible for recognition of the promoter -35 element, σ^{70} region 4 ($\sigma R4$; pink in Fig 3b [59,60]).

The AR1- α CTD interaction recruits α CTD to the DNA segment immediately upstream of the DNA site for CAP, with α CTD-DNA interaction being mediated by the “265 determinant” of α CTD (red in Fig 3b [1,55]) and the DNA minor groove [61,62,63] and thereby recruits RNAP to the promoter to form the RNAP-promoter closed complex (Fig 3b [56,64]). The AR2- α NTD and AR3- $\sigma R4$ interactions activate transcription through a post-recruitment mechanism, facilitating isomerization of the RNAP-promoter closed complex to yield the RNAP-promoter open complex [56,58,64].

As noted above, RNAP contains two protomers of α CTD: α CTD^I and α CTD^{II} (Fig 3b [29]). At Class II CAP-dependent promoters, one α CTD protomer--either α CTD^I or α CTD^{II}, but preferably α CTD^I--interacts with CAP [1,41,42]. The other α CTD protomer interacts non-specifically with upstream DNA [1,62,63].

Figure 5b presents a structural model of the *intact, full* Class II CAP-RNAP-promoter complex at CC(-41.5). The model was constructed in three steps: (i) joining the crystal structures of the CAP- α CTD-DNA complex [44**], the $\sigma R4$ -(-35 element) complex [47**], and the RNAP-DNA complex [49**]--superimposing DNA segments of the three structures onto a single, continuous DNA segment having a site for α CTD, a site for CAP, a -35 element, and a -10 element, spaced as at CC(-41.5); (ii) refining local DNA-helix parameters in the downstream half of the DNA site for CAP (positions -33 to -38), using experimentally defined AR2- α NTD^I interactions [41,56], AR3- $\sigma R4$ interactions [58,59,60], and non-interpenetration as constraints, and using sequence-dependent DNA-deformation energies [50] as restraints; and (iii) modelling downstream DNA segments as in published models of the RNAP-promoter open complex [39,51**]. In step (ii), in order to satisfy the refinement constraints, it was necessary to introduce a relatively large change in local DNA-helix parameters in the downstream half-site of the DNA site for CAP: specifically, it was necessary to replace the primary kink within the downstream half of the DNA site for CAP ($\sim 40^\circ$ roll at the $-34/-35$ base-pair step) by a smooth bend ($\sim 10^\circ$ roll distributed over the $-34/-35$, $-35/-36$, and $-36/-37$ base-pair steps, yielding a local DNA geometry reminiscent of that observed in two structures of CAP-DNA complexes (see above [22*,24]). The resulting model is consistent with all available experimental information and provides an indispensable structural framework for understanding Class II CAP-dependent transcription. One important feature of the model is the proposed proximity between the DNA minor grooves at positions -73 and -83 and α CTD (Fig 5b). The proposed proximity accounts for results indicating that the α CTD protomer not in contact with CAP interacts with the DNA minor groove at positions -73 and -83 [62,63] and is available in principle to interact with a second activator in the -93 , or -103 region [42,62,63]. Another important feature of the model is the proposed requirement for restructuring of DNA local geometry in the downstream half of the DNA site for CAP (conversion of the primary kink to a smooth bend) in order to permit formation of AR2- α NTD^I and AR3- $\sigma R4$ interactions. The proposed requirement for restructuring provides a possible explanation for the observation that AR2- α NTD^I and AR3- $\sigma R4$ interactions do not facilitate binding of RNAP to the promoter to yield the RNAP-promoter closed complex, but do facilitate isomerization of the RNAP-promoter closed complex to yield the RNAP-promoter open complex [56,58,64]: i.e., restructuring, and formation of restructuring-dependent AR2- α NTD^I and AR3- $\sigma R4$ interactions, may occur only during isomerization of the RNAP-promoter closed complex to yield the RNAP-promoter open complex.

Prospect

DNA binding and transcription activation by CAP should be amenable to a complete structural description. Priorities for future work include: (i) determination of high-resolution structures of CAP-DNA complexes having all possible base-pair steps at the primary-kink site (structures relevant to DNA bending and Class II CAP-dependent transcription), (ii) determination of high-resolution structures of the CAP- α CTD- σ R4-DNA and CAP- σ R4-DNA complexes (structures spanning the upstream-promoter and core-promoter regions of the Class I and Class II CAP-RNAP-promoter complexes), and (iii) determination of low-resolution structural envelopes or high-resolution structures of intact Class I and Class II CAP-RNAP-promoter complexes. Based on recent experience, progress is likely to be rapid.

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Abbreviations

CAP	catabolite activator protein
AR1, AR2, AR3	activating region 1, activating region 2, activating region 3
RNAP	RNA polymerase holoenzyme ($\alpha^I\alpha^{II}\beta\beta'\omega\sigma^{70}$)
αNTD	RNAP α subunit N-terminal domain
αCTD	RNAP α subunit C-terminal domain
σR2, σR3.1, σR4	σ^{70} region 2, σ^{70} region 3.1, σ^{70} region 4

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promoters], defines the determinants of α CTD and σ^{70} required for the interaction, and presents a structural model for the interaction. The α CTD- σ^{70} interaction spans the upstream promoter and core promoter, thereby linking recognition of upstream-promoter-elements and activators in the upstream promoter with recognition of the -35 element in the core promoter

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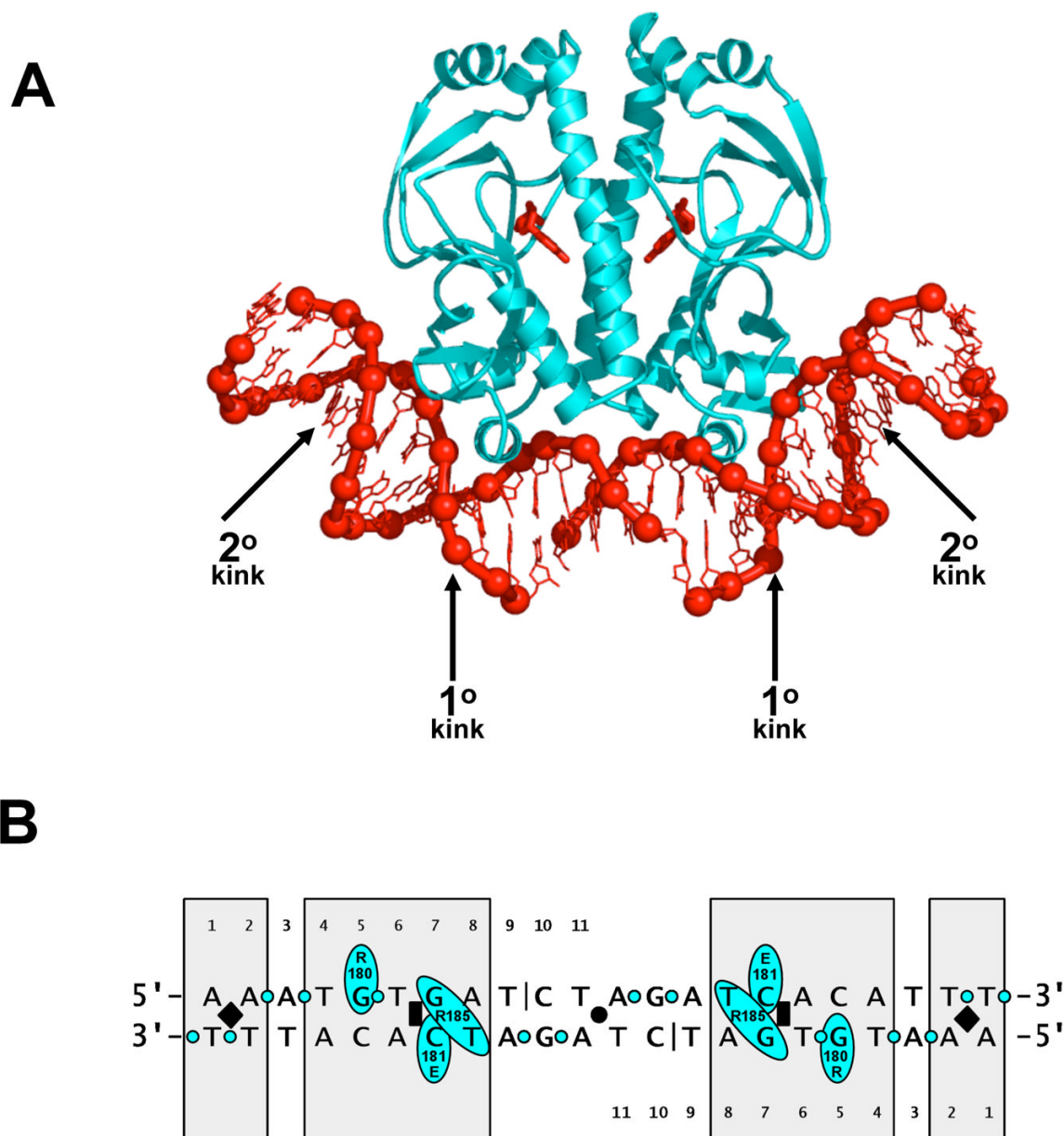


Figure 1.

DNA binding by CAP: structure of the CAP-DNA complex.

(A) Structure of CAP in complex with its consensus DNA site (PDB 1RUN) [14], showing primary- and secondary-kink sites. CAP is in cyan; DNA and cAMP bound to CAP are in red. The crystallization DNA fragment contained a single-phosphate gap between positions 9 and 10 of each DNA half-site (Fig 1b).

(B) Summary of CAP-DNA interactions. Shaded boxes indicate positions at which CAP exhibits strong sequence preferences [11,15,16,17]. The black circle, black rectangles, and black diamonds indicate, respectively, the two fold-symmetry axis, the primary-kink sites; and the secondary-kink sites. The black vertical lines indicate the positions of single-phosphate gaps present in the crystallization DNA fragment. The cyan ovals and cyan circles indicate, respectively, amino acid-base contacts and amino acid-phosphate contacts.

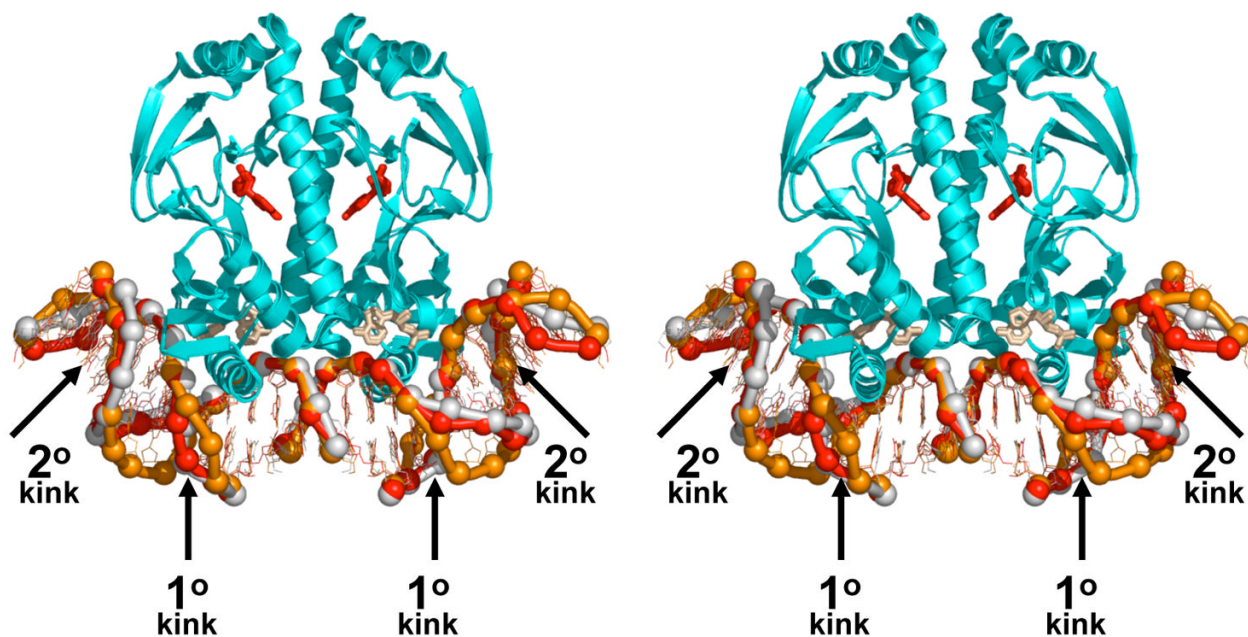
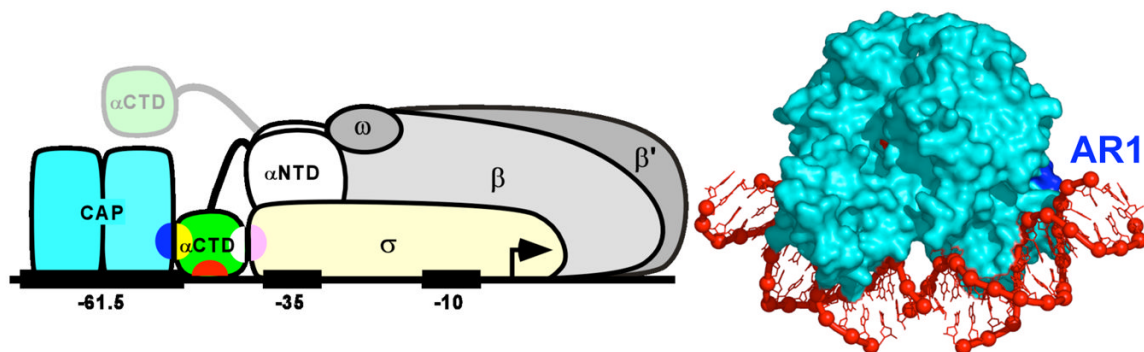


Figure 2. DNA binding by CAP: structures of CAP-DNA complexes with substitutions in the primary-kink site. Superimposed structures of CAP in complex with the consensus DNA site (DNA in red; PDB 1O3Q [23*]), CAP in complex with DNA having C:G in place of T:A at position 6 of each DNA half-site (DNA in yellow; PDB 1O3R [23*]), and [Asp181]CAP in complex with DNA having C:G in place of T:A at position 6 of each DNA half-site (DNA in orange; PDB 1O3S [22*]). The structures were obtained from isomorphous crystals with space-group symmetry $P3_121$. Structures of CAP-DNA complexes with this space-group symmetry exhibit two molecules of cAMP per CAP subunit: one in the high-affinity site for cAMP (red), and one in the low-affinity site for cAMP (beige) [22*,23*,24].

A Class I



B Class II

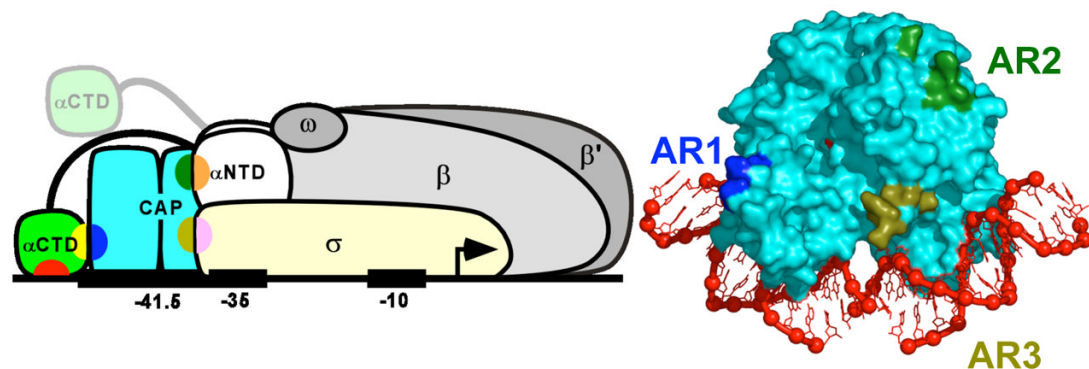


Figure 3.

Transcription activation by CAP: schematic models and activating regions.

(A) Transcription activation at a Class I CAP-dependent promoter [1,40**]. Left: Ternary complex of CAP, RNAP, and a Class I CAP-dependent promoter having the DNA site for CAP centered at position -61.5 [e.g., *lac* or CC(-61.5)]. Transcription activation involves interaction between AR1 of the downstream subunit of CAP (blue) and the “287 determinant” of one α CTD protomer (yellow). The AR1- α CTD interaction facilitates binding of α CTD, through its “265 determinant” (red), to the DNA segment immediately downstream of CAP and, through its “261 determinant” (white), to residues 573–604 within σ R4 (pink). The second α CTD protomer (positioned arbitrarily in figure) interacts non-specifically with upstream DNA [1, 39,41]. Right: Structure of the CAP-DNA complex showing AR1 of the downstream subunit (blue).

(B) Transcription activation at a Class II CAP-dependent promoter [1,58,59]. Left: Ternary complex of CAP, RNAP, and a Class II CAP-dependent promoter having the DNA site for CAP centered at position -41.5 [e.g., *gal* or CC(-41.5)]. Transcription activation involves three sets of CAP-RNAP interactions: (i) interaction between AR1 of the upstream subunit of CAP (blue) and the “287 determinant” of one α CTD (yellow), an interaction that facilitates binding of α CTD, through its “265 determinant” (red), to the DNA segment immediately upstream of CAP; (ii) interaction between AR2 of the downstream subunit of CAP (dark green) and residues 162–165 of α NTD^I (orange); and (iii) interaction between AR3 of the downstream subunit of CAP (olive green) and residues 593–603 of σ R4 (pink). The second α CTD protomer (positioned arbitrarily in figure) interacts non-specifically with upstream DNA [1,62,63].

Right: Structure of the CAP-DNA complex showing AR1 of the upstream subunit (blue), AR2 of downstream subunit (dark green), and AR3 of downstream subunit (olive green).

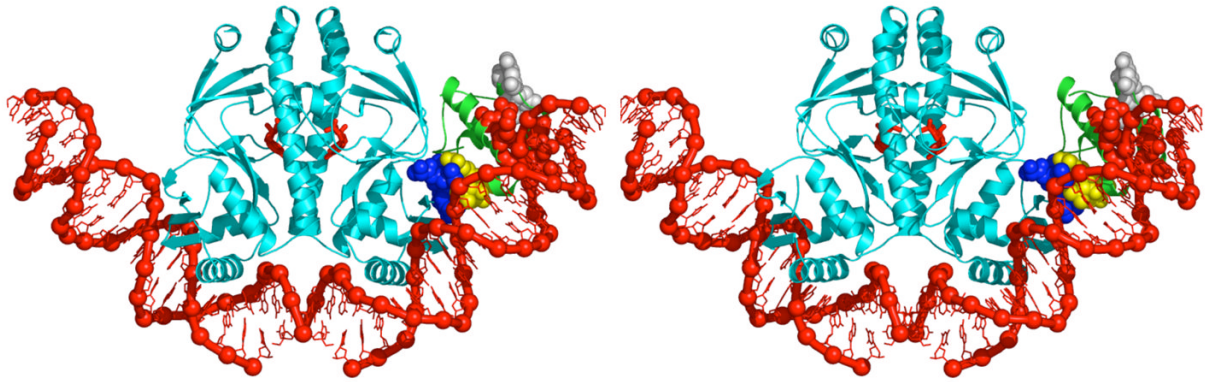


Figure 4. Transcription activation by CAP: structure of the CAP- α CTD-DNA complex. CAP- α CTD-DNA interactions representative of those at Class I and Class II CAP-dependent promoters (PDB 1LB2 [44**]). CAP is in cyan; α CTD is in green; DNA and cAMP bound to CAP are in red. AR1 of CAP (blue), the “287 determinant” of α CTD (yellow), the “265 determinant” of α CTD (red), and the “261 determinant” of α CTD (white) are in van der Waals representations.

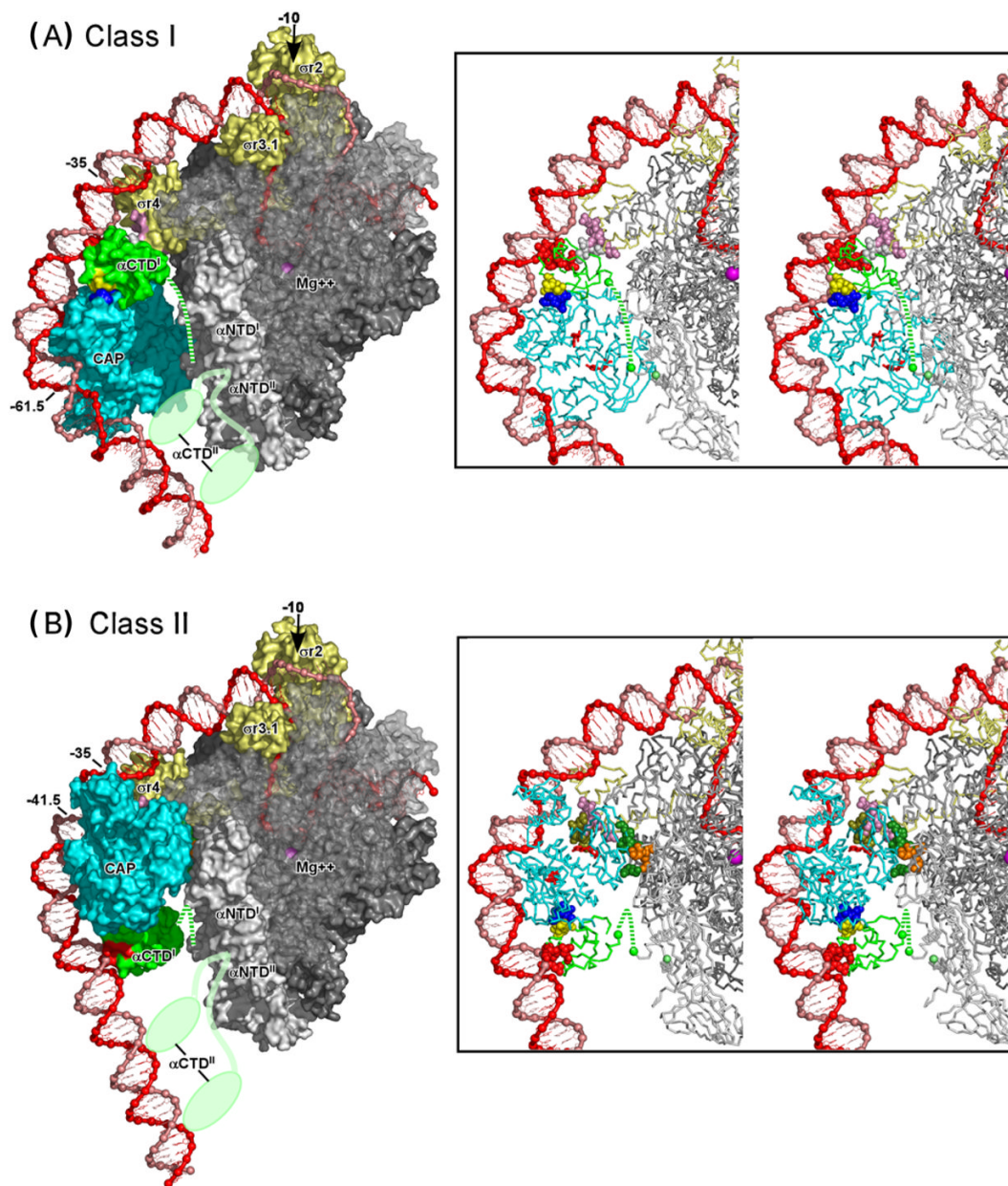


Figure 5. Transcription activation by CAP: structural models of intact Class I and Class II CAP-RNAP-promoter complexes.

(A) Structural model of the intact Class I CAP-RNAP-promoter complex at *lac*.

(B) Structural model of the intact Class I CAP-RNAP-promoter complex at CC(-41.5).

In each panel, a molecular surface representation is shown at left; and a stereodiagram with a ribbon representation is shown at right. Colors of CAP and RNAP are as in Fig 3: CAP is in cyan; α CTD^I is in green; α CTD^{II} is in light green (shown in two alternative positions in surface representations; omitted for clarity in ribbon representations); σ^{70} is in light yellow; α NTD^I and α NTD^{II} are in light gray; β is in medium gray (semi-transparent in surface representations, to permit view of DNA strands in RNAP active-center cleft); and β' and ω are in dark gray. Colors of determinants of CAP and RNAP also are as in Fig 3: AR1, AR2, and AR3 of CAP

are in dark blue, dark green, and olive green; the 287, 265, and 261 determinants of α CTD^I are in yellow, red, and white; the 162–165 determinant of α NTD^I is in orange; and the 593–604 determinant of σ^{70} is in pink. The DNA template and nontemplate strands are in red and pink. The C-terminus of α NTD^I (green) the C-terminus of α NTD^{II} (light green), and the active-center Mg^{++} (magenta) are indicated by spheres. The linker connecting α CTD^I and α NTD^I is indicated by a dashed green line. The linker connecting α CTD^{II} and α NTD^{II} is indicated in each of two alternative positions as a light green line.

Methods: Models were constructed by: (i) joining crystal structures of the CAP- α CTD-DNA complex (PDB 1LB2 [44**]), the σ R4-(−35 element) complex (PDB 1KU7 [47**]), and an RNAP-DNA complex (PFB 1L9Z [49**]; residues 150–160 and 164–170 of α NTD^I modelled as in PDB 1BDF [65]; residues 161–163 of α NTD^{II} modelled along shortest sterically allowed path; side chains modelled using MaxSprout [<http://www.ebi.ac.uk/maxsprout/>])--superimposing DNA segments of the three structures onto a single, continuous DNA segment having sites spaced as at *lac* (panel A) or CC(−41.5) (panel B); (ii) deforming conformations of DNA positions −13 to −31 and −41 to −36 (panel A) or −13 to −30 and −38 to 33 (panel B) to minimize the elastic energy of DNA at the base-pair level [50] while satisfying DNA anchoring conditions, non-interpenetration constraints (C^{α} - C^{α} distance ≥ 3.5 Å for all residue pairs), and proximity constraints (C^{α} - C^{α} distance ≤ 12 Å for residue pairs specified below); and (iii) modelling DNA template-strand positions −11 to +20 and nontemplate-strand positions −7 to +20 as in published models of the RNAP-promoter open complex [39,51**]. For panel A, the following proximity constraints were used: proximity of residues 257, 258, 259, and 261 of α CTD to at least one of residues 593, 596, 597, 600, 601, and 604 of σ R4, and vice versa (mutational analysis [36,37,40**]); and proximity of residue 261 of α CTD to residues 596 and 600 of σ R4 (suppression analysis [40**]) (residues numbered as in *E. coli* RNAP). For panel B, the following proximity constraints were used: proximity of residues 19, 21, 96, and 101 of the downstream CAP subunit to at least one of residues 162, 163, 164, and 165 of α NTD^I, and vice versa (mutational analysis [56]); proximity of residues 52, 53, 54, 55, and 58 of the downstream CAP subunit to at least one of residues 593, 596, 597, 599, and 603 of σ R4 and vice versa (mutational analysis [58,60]); and proximity of residue 58 of the downstream CAP subunit to residue 596 of σ R4 (suppression analysis [59]) (residues numbered as in *E. coli* RNAP). The models have been deposited in the PDB (PDB **** and ****). Figures were prepared using PyMol [<http://www.pymol.org>]. The view orientation reflects rotation by −45 on the y-axis relative to the “upstream” view orientation in published models of the RNAP-promoter open complex [39,51**]).