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Catabolite activator protein: DNA binding and transcription activation

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Recently determined structures of the *Escherichia coli* catabolite activator protein (CAP) in complex with DNA, and in complex with the RNA polymerase α subunit C-terminal domain (αCTD) and DNA, have yielded insights into how CAP binds DNA and activates transcription. Comparison of multiple structures of CAP–DNA complexes has revealed the contributions of direct and indirect readout to DNA binding by CAP. The structure of the CAP–αCTD–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–αCTD–DNA complex, the structure of an RNA polymerase–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.

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Abbreviations

αCTD	RNAP α subunit C-terminal domain
αNTD	RNAP α subunit N-terminal domain
AR1, AR2, AR3	activating region 1, activating region 2, activating region 3
bp	base pairs
CAP	catabolite activator protein
FRET	fluorescence resonance energy transfer
PDB	Protein Data Bank
RNAP	RNA polymerase holoenzyme
σR2, σR3.1, σR4	σ^{70} region 2, σ^{70} region 3.1, σ^{70} region 4

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 [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 its three-dimensional structure determined, and CAP has been the subject of extensive biophysical, biochemical and genetic investigations.

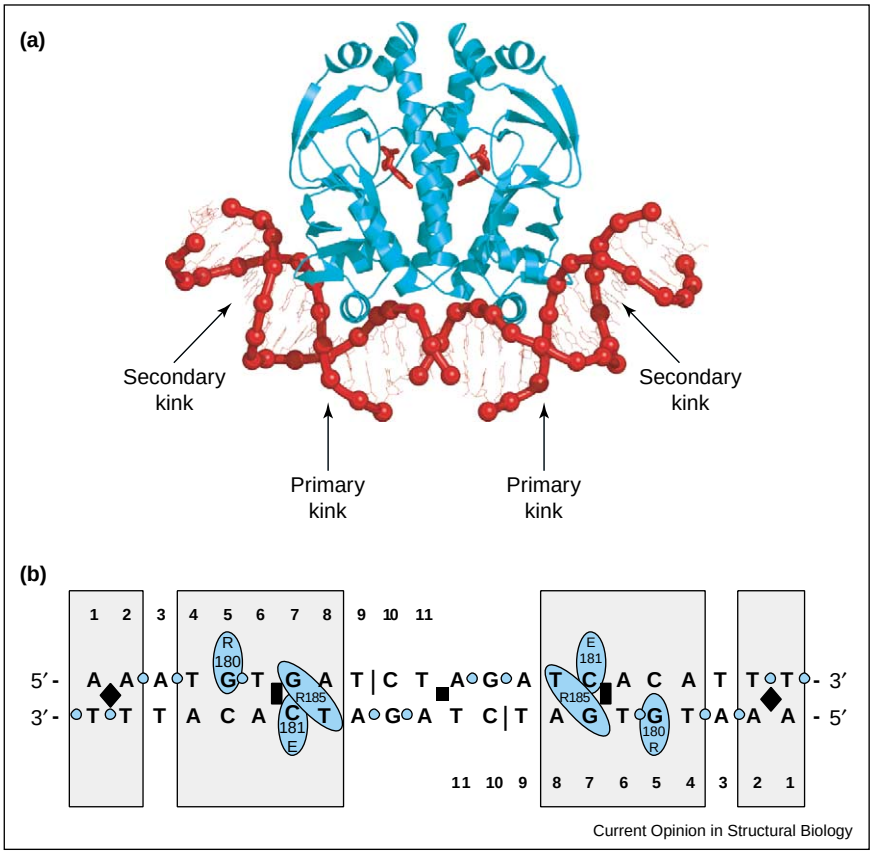
Transcription activation by CAP at the simplest CAP-dependent promoters requires only three macromolecular components — CAP, RNAP and promoter DNA — and only one DNA site for CAP [1]. Transcription activation by CAP at such promoters is simpler than most examples of transcription activation in bacteria (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 other examples of transcription activation.

The immediate scope of this article is to review the 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 the 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 twofold-symmetric DNA site: 5′-AATGTGATCTA-GATCACATTT-3′ [11]. The CAP–DNA complex is

Figure 1



DNA binding by CAP — structure of the CAP–DNA complex. **(a)** Structure of CAP in complex with its consensus DNA site (PDB code 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 (see Figure 1b). **(b)** Summary of CAP–DNA interactions. Shaded boxes indicate positions at which CAP exhibits strong sequence preferences [11,15–17]. The black circle, black rectangles and black diamonds indicate, respectively, the twofold-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.

twofold symmetric: one subunit of CAP interacts with one half of the DNA site and the other subunit of CAP interacts with the other half of the DNA site (Figure 1) [12–14]. Initial structures of CAP–DNA complexes revealed two distinctive features [12–14]. First, CAP recognizes its DNA site through a combination of 'direct readout' (DNA sequence recognition mediated by direct hydrogen-bond 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 approximately 80°, wrapping the 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 approximately 40°, compressing the DNA major groove between positions 6 and 7, and a 'secondary kink' of approximately 9°, compressing the DNA minor groove between positions −1 and 2. Recent crystallographic and biophysical studies have shed further light on these issues.

Direct versus indirect recognition

CAP exhibits strong sequence preferences at seven positions within each DNA half-site: positions 1, 2 and 4–8 (boxed positions in Figure 1b) [11,15–17]. Sequence preferences at three of these positions within each DNA half-site — positions 5, 7 and 8 — are accounted for by direct amino acid–base contacts (Figure 1b) [12–14,18–20]. The guanidinium sidechain of Arg180 forms hydrogen bonds with the guanine O^6 and N7 atoms of the consensus base pair G•C at position 5; the carboxylate sidechain of Glu181 forms a hydrogen bond with the cytosine N^4 atom of the consensus base pair G•C at position 7; and the guanidinium sidechain of Arg185 forms a hydrogen bond with the thymine O^4 atom of the consensus base pair A•T at position 8 (and, in some structures, also with the guanine O^6 and/or N7 atoms of

G•C at position 7). By contrast, sequence preferences at the remaining positions occur in the absence of amino acid–base contacts and thus must involve indirect readout (Figure 1b) [12,13].

DNA kinking and indirect recognition

The primary kink is located between positions 6 and 7 (Figure 1b). Positions 6 and 7 are part of a T•A/G•C base-pair step, a base-pair step 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 the formation of the DNA kink between positions 6 and 7, and the 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 Figure 2) [22]. These results provide strong support for the proposed connection between specificity for T•A at position 6 and formation of the primary kink [22].

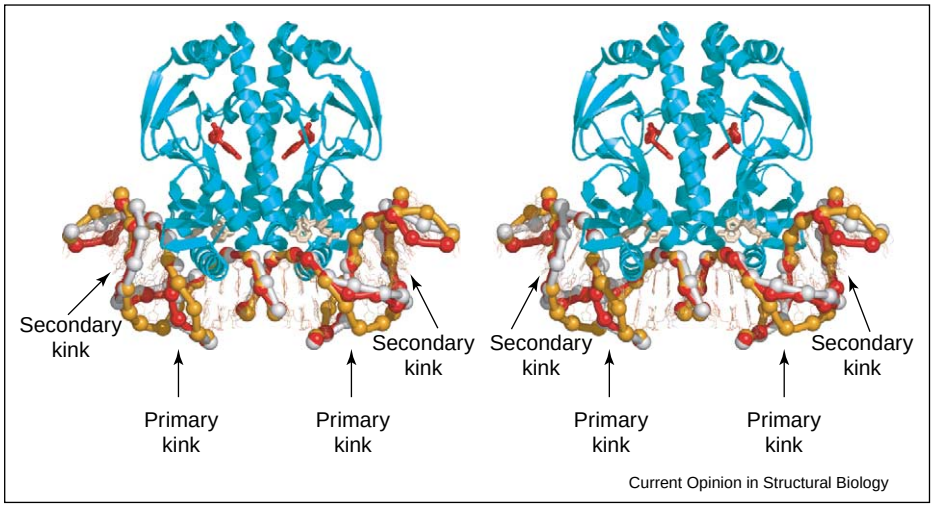
A companion study showed that complexes of CAP with DNA sites having the consensus base pair T•A or the nonconsensus base pair C•G at position 6 exhibit similar overall DNA bend angles and local geometries of DNA kinking (DNA structures in yellow and red in Figure 2) [23]. These results suggest that 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 to make a definitive statement.)

The results of both studies imply that the main determinant of 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 the structure of an [Asp181]CAP–DNA complex (wherein [Asp181]-CAP is a CAP derivative with a Glu181→Asp181 substitution, a substitution that shortens the residue 181 sidechain by one methylene group) — including even the hydrogen bond between the sidechain carboxylate of residue 181 and the cytosine N^4 atom of G•C at position 7 [22]. The overall DNA bend angles also were shown to be similar (DNA structures in white and red in Figure 2) [22]. However, the local DNA geometries at the primary kink site 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 Figure 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

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 code 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 white; PDB code 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 code 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) [22–24].

protein–DNA interaction, and not DNA sequence — with the protein in essence 'bending DNA to its will'.

We note, however, complexity in the kinking versus bending story. Two nearly isomorphous structures of wild-type CAP–DNA complexes solved in space group $P3_121$ 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 length (38 bp versus 46 bp), sequence (consensus versus nonconsensus at positions 1, 9, and 10 of each half-site) and position of single-phosphate gaps (top strand versus bottom strand at position 10 of each half-site). Therefore, in this case, it is not possible to deduce the basis of the different local DNA helical parameters at the primary kink site.

DNA bending in solution

Nanosecond time-resolved fluorescence resonance energy transfer (FRET) measurements between a probe incorporated in CAP and a complementary probe incorporated in each of a series of sites in the DNA indicate that the mean DNA bend angle in the CAP–DNA complex in solution is $77(\pm3)^\circ$ — a value consistent with the mean DNA bend angle observed in crystal structures, $80(\pm12)^\circ$ [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 approximately 100° [25].

What factors are responsible for DNA bending by CAP? One factor is the 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 [26–28]. 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 40–50° [27].

Another, probably more important, factor is the 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: by stabilizing the bent state through amino acid–phosphate interactions [12,15,25,27], and 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 $\sim5^\circ$ per residue per half-complex, as assessed in FRET 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 of 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 RNAP (subunit composition $\alpha^{I}\alpha^{II}\beta\beta'\omega\sigma^{70}$ [29]), and proceeds through a simple 'recruitment' mechanism, whereby CAP facilitates the binding of RNAP to the promoter to yield the RNAP–promoter closed complex (Figure 3a) [1].

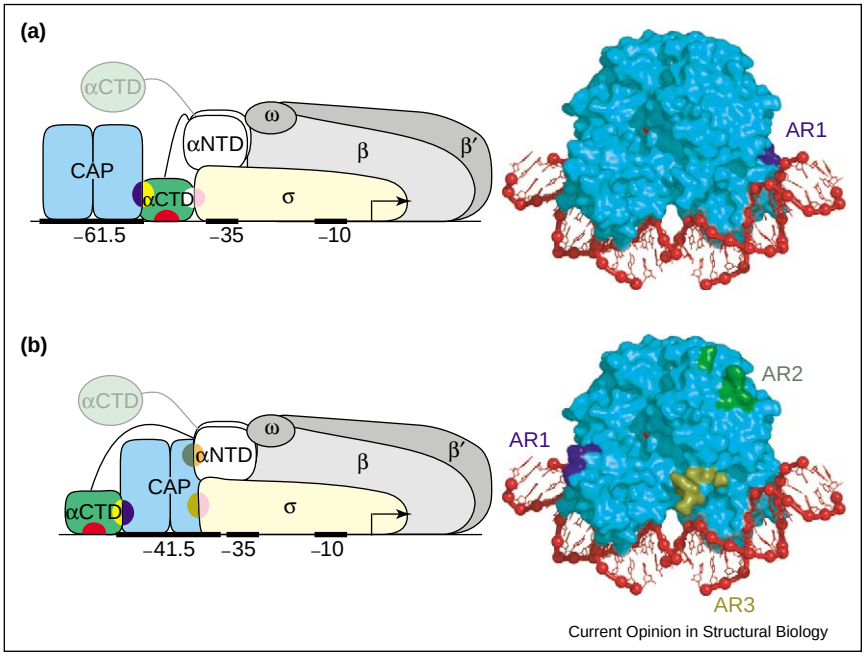
At class II CAP-dependent promoters, the DNA site for CAP overlaps the core promoter, overlapping the core-promoter −35 element. The best-characterized class II CAP-dependent promoters are the *galP1* promoter and the artificial promoter CC(−41.5), each of which has a DNA site for CAP centered at position −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 facilitates both the binding of RNAP to the promoter to yield the RNAP–promoter closed complex and isomerization of the RNAP–promoter closed complex to yield the RNAP–promoter open complex (Figure 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 (αCTD), an 85 amino acid independently folded domain that is flexibly tethered to the remainder of RNAP (Figure 3a) [1,30]. Interaction of CAP with αCTD facilitates the binding of αCTD — 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 αCTD is mediated by 'activating region 1' of the downstream subunit of CAP (AR1;

Figure 3

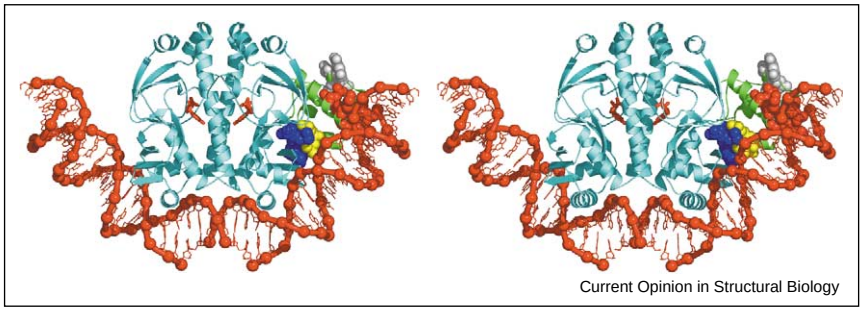


Transcription activation by CAP — schematic models and activating regions. **(a)** Transcription activation at a class I CAP-dependent promoter [1,40••]. Left: ternary complex of CAP, RNAP and a class I CAP-dependent promoter having the DNA site for CAP centered at position −61.5 [e.g. *lac* or CC(−61.5)]. Transcription activation involves interaction between AR1 of the downstream subunit of CAP (blue) and the '287 determinant' of one αCTD protomer (yellow). The AR1–αCTD interaction facilitates the binding of αCTD, through its '265 determinant' (red), to the DNA segment immediately downstream of CAP and, through its '261 determinant' (white), to residues 573–604 within σR4 (pink). The second αCTD protomer (positioned arbitrarily in figure) interacts nonspecifically 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: interaction between AR1 of the upstream subunit of CAP (blue) and the '287 determinant' of one αCTD (yellow), an interaction that facilitates the binding of αCTD, through its '265 determinant' (red), to the DNA segment immediately upstream of CAP; interaction between AR2 of the downstream subunit of CAP (dark green), and residues 162–165 of αNTD^{I} (orange); and interaction between AR3 of the downstream subunit of CAP (olive green), and residues 593–603 of σR4 (pink). The second αCTD protomer (positioned arbitrarily in figure) interacts nonspecifically with upstream DNA [1,62,63]. Right: structure of the CAP–DNA complex showing AR1 of the upstream subunit (blue), AR2 of the downstream subunit (dark green), and AR3 of the downstream subunit (olive green).

blue in Figure 3a) [31–35] and the '287 determinant' of αCTD (yellow in Figure 3a) [36]. Biochemical and genetic results further indicate that the interaction between αCTD and DNA is mediated by the '265 determinant' of αCTD (red in Figure 3a) [36–38] and the DNA minor groove [39]. At class I CAP-dependent promoters, such as the *lac* and CC(−61.5) promoters, where the DNA site for CAP is centered at position −61.5, interaction between CAP and αCTD places αCTD adjacent to σ^{70}, and permits functional protein–protein interaction between αCTD and σ^{70} (Figure 3a) [40••]. The interaction between αCTD and σ^{70} is mediated by the '261 determinant' of αCTD (white in Figure 3a) [36,37,40••] and residues 573–604 within the module of σ^{70} responsible for recognition of the promoter −35 element, σ^{70} region 4 (σR4; pink in Figure 3a) [40••]. RNAP contains two copies of αCTD: αCTD^{I} and αCTD^{II} (Figure 3a) [29]. At class I CAP-dependent promoters, one αCTD protomer — interchangeably αCTD^{I} or αCTD^{II} — interacts with CAP [1,39,41,42]. The other αCTD protomer interacts nonspecifically with upstream DNA [1,39,43].

The recently determined crystal structure of a complex containing CAP, αCTD, 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 (Figure 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 αCTD, and αCTD makes protein–DNA interactions with the DNA minor groove adjacent to the DNA site for CAP. The interaction between CAP and αCTD is mediated by AR1 of CAP and the 287 determinant of αCTD (blue and yellow in Figures 3a and 4). The interaction between αCTD and DNA is mediated by the 265 determinant of αCTD (red in

Figure 4



Transcription activation by CAP — structure of the CAP–αCTD–DNA complex. CAP–αCTD–DNA interactions representative of those at class I and class II CAP-dependent promoters (PDB code 1LB2 [44••]). CAP is in cyan; αCTD is in green; DNA and cAMP bound to CAP are in red. AR1 of CAP (blue), the '287 determinant' of αCTD (yellow), the '265 determinant' of αCTD (red), and the '261 determinant' of αCTD (white) are in van der Waals representations.

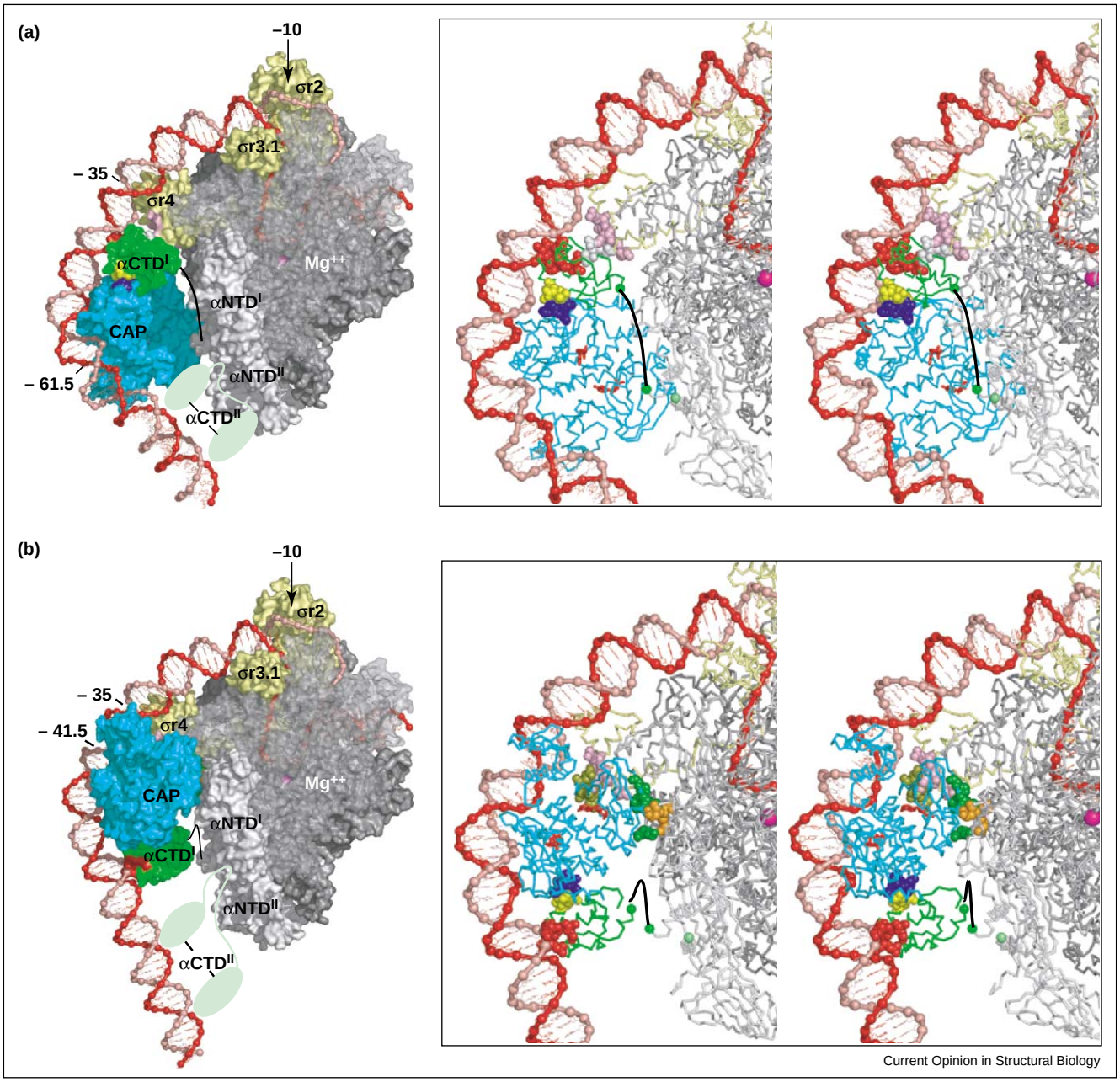
Figures 3a and 4), and the backbone and spine of hydration of the DNA minor groove adjacent to the DNA site for CAP. The 261 determinant of αCTD (white in Figures 3a and 4) is located on the face of αCTD opposite from CAP and is prominently exposed, consistent with availability to participate in interactions with σR4.

Significantly, in the structure of the CAP–αCTD–DNA complex, there are no conformational changes in CAP and αCTD, and the interface between CAP and αCTD is small (six residues each of CAP and αCTD; 630 $Å^2$ 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 the interaction of the general transcription machinery with promoter DNA [1,30,44••,45,46]. (Activation by recruitment does not require conformational signaling 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–αCTD–DNA complex [44••] and the σR4–(−35 element) complex [47••] — simply superimposing DNA segments of the two structures to generate a single, continuous DNA segment having a site for CAP, a site for αCTD, 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–αCTD–σR4–DNA complex at a class I CAP-dependent promoter, such as *lac* or CC(−61.5) [40••,48••]. The resulting model places the 261 determinant of αCTD adjacent to the 573–604 determinant of σR4, permitting favorable electrostatic interaction between the determinants (which have, respectively, high net negative charge and high net positive charge) and, with modest adjustment of sidechain torsion angles, direct contact between experimentally defined interacting residues. The fit between modeled 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–αCTD–DNA complex [44••], the σR4–(−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 αCTD, 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 αCTD–σR4 interactions [40••] and noninterpenetration as constraints, and using sequence-dependent DNA deformation energies [50] as restraints; and (iii) modeling 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 segment 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 groove at positions −43, −53,

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 II CAP–RNAP–promoter complex at CC(−41.5). In each panel, a molecular surface representation is shown at left and a stereo diagram with a ribbon representation is shown at right. CAP is in cyan; αCTD^{I} is in green; αCTD^{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; αNTD^{I} and αNTD^{II} are in light gray; β is in medium gray (semi-transparent in surface representations to permit view of DNA strands in the RNAP active center cleft); and β′ and ω are in dark gray. AR1, AR2, and AR3 of CAP are in dark blue, dark green, and olive green, respectively; the 287, 265, and 261 determinants of αCTD^{I} are in yellow, red, and white, respectively; the 162–165 determinant of αNTD^{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, respectively. The C terminus of αNTD^{I} (green), the N terminus of αCTD^{I} (green), the C terminus of αNTD^{II} (light green), and the active center Mg^{2+} (magenta) are indicated by spheres. The linker connecting αCTD^{I} and αNTD^{I} is indicated by a black line [αNTD^{I}–αCTD^{II} distance = 37 Å in (a) and 21 Å in (b)]. The linker connecting αCTD^{II} and αNTD^{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–αCTD–DNA complex (PDB code 1LB2 [44••]), the σR4–(−35 element) complex (PDB code 1KU7 [47••]), and an RNAP–DNA complex (PDB code 1L9Z [49••]; residues 150–160 and 164–170 of αNTD^{I} modeled as in PDB entry 1BDF [65]; residues 161–163 of αNTD^{I} modeled along shortest sterically allowed path; sidechains modeled using MaxSprout [http://www.ebi.ac.uk/maxsprout/]) — superimposing DNA segments of the three structures to generate a single, continuous DNA segment having sites spaced as at *lac* (a) or CC(−41.5) (b); (ii) deforming conformations of DNA

−63, −73, −83, and −93 is proposed to be in proximity to αCTD^{I} and αCTD^{II} (Figure 5a). The proposed positions of the upstream promoter DNA minor grooves relative to αCTD^{I} and αCTD^{II} account for results indicating that the αCTD protomer not in contact with CAP can be cross-linked 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 (Figure 3b) [1]. AR1 of the upstream subunit of CAP (blue in Figure 3b) [34,35,53,54] interacts with the '287 determinant' of αCTD (yellow in Figure 3b) [55]. Activating region 2 of the downstream subunit of CAP (AR2; dark green in Figure 3b) [56] interacts with residues 162–165 within the RNAP α^{I} N-terminal domain (αNTD^{I}) (orange in Figure 3b) [1,41,56]. AR3 of the downstream subunit of CAP (olive green in Figure 3b) [53,54,56–59] interacts with residues 593–603 within the module of σ^{70} responsible for recognition of the promoter −35 element, σR4 (pink in Figure 3b) [59,60].

The AR1–αCTD interaction recruits αCTD to the DNA segment immediately upstream of the DNA site for CAP, with the αCTD–DNA interaction being mediated by the '265 determinant' of αCTD (red in Figure 3b) [1,55] and the DNA minor groove [61–63], and thereby recruits RNAP to the promoter to form the RNAP–promoter closed complex (Figure 3b) [56,64]. The AR2–αNTD and AR3–σR4 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 αCTD: αCTD^{I} and αCTD^{II} (Figure 3b) [29]. At class II CAP-dependent promoters, one αCTD protomer — either αCTD^{I} or αCTD^{II}, but preferably αCTD^{I} — interacts with CAP [1,41,42,62]. The other αCTD protomer interacts nonspecifically 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–αCTD–DNA complex [44••], the σR4–(−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 αCTD, 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–αNTD^{I} interactions [41,56], AR3–σR4 interactions [58–60], and noninterpenetration as constraints, and using sequence-dependent DNA deformation energies [50] as restraints; and (iii) modeling downstream DNA segments as in published models of the RNAP–promoter open complex [39,51••]. In step (ii), to satisfy the refinement constraints, it was necessary to introduce a relatively large change in the local DNA helix parameters in the downstream half 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 (∼40° roll at the −34/−35 base-pair step) with a smooth bend (∼10° 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 of the DNA minor groove at positions −73 and −83 and αCTD (Figure 5b). The proposed proximity accounts for results indicating that the αCTD 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 the local DNA geometry in the downstream half of the DNA site for CAP (conversion of the primary kink to a smooth bend) to permit formation of AR2–αNTD^{I} and AR3–σR4 interactions. The proposed requirement for restructuring provides a possible explanation for the observation that the

(Figure 5 Legend Continued) positions −13 to −31 and −41 to −36 (a), or −13 to −30 and −38 to −33 (b) to minimize the elastic energy of DNA at the base-pair level [50], while satisfying DNA anchoring conditions, noninterpenetration 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) modeling 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••]. In (a), the following proximity constraints were used: proximity of residues 257, 258, 259, and 261 of αCTD to at least one of residues 593, 596, 597, 600, 601, and 604 of σR4, and vice versa (mutational analysis [36,37,40••]); and proximity of residue 261 of αCTD to residues 596 and 600 of σR4 (suppression analysis [40••]) (residues numbered as in *E. coli* RNAP). In (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 αNTD^{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 σR4, and vice versa (mutational analysis [58,60]); and proximity of residue 58 of the downstream CAP subunit to residue 596 of σR4 (suppression analysis [59]) (residues numbered as in *E. coli* RNAP). 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••].

AR2–αNTD^I and AR3–$\sigma R4$ interactions do not facilitate binding of RNAP to the promoter to yield the RNAP–promoter closed complex, but rather facilitate isomerization of the RNAP–promoter closed complex to yield the RNAP–promoter open complex [56,58,64]: i.e. restructuring, and the formation of restructuring-dependent AR2–αNTD^I and AR3–$\sigma R4$ interactions, may occur only during isomerization of the RNAP–promoter closed complex to yield the RNAP–promoter open complex.

Conclusions

DNA binding and transcription activation by CAP should be amenable to a complete structural description. Priorities for future work include: determination of high-resolution structures of CAP–DNA complexes with all possible base-pair steps at the primary kink site (structures relevant to DNA bending and class II CAP-dependent transcription); determination of high-resolution structures of the CAP–αCTD–$\sigma R4$–DNA and CAP–$\sigma R4$–DNA complexes (structures spanning the upstream promoter and core promoter regions of the class I and class II CAP–RNAP–promoter complexes); and 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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