Transcription Activation by Catabolite Activator Protein (CAP)

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Transcription activation by Escherichia coli catabolite activator protein (CAP) at each of two classes of simple CAP-dependent promoters is understood in structural and mechanistic detail. At class I CAP-dependent promoters, CAP activates transcription from a DNA site located upstream of the DNA site for RNA polymerase holoenzyme (RNAP); at these promoters, transcription activation involves protein-protein interactions between CAP and the RNAP α subunit C-terminal domain that facilitate binding of RNAP to promoter DNA to form the RNAP-promoter closed complex. At class II CAP-dependent promoters, CAP activates transcription from a DNA site that overlaps the DNA site for RNAP; at these promoters, transcription activation involves both: (i) protein-protein interactions between CAP and RNAP α subunit C-terminal domain that facilitate binding of RNAP to promoter DNA to form the RNAP-promoter closed complex; and (ii) protein-protein interactions between CAP and RNAP α subunit N-terminal domain that facilitates isomerization of the RNAP-promoter closed complex to the RNAP-promoter open complex. Straightforward combination of the mechanisms for transcription activation at class I and class II CAP-dependent promoters permits synergistic transcription activation by multiple molecules of CAP, or by CAP and other activators. Interference with determinants of CAP or RNAP involved in transcription activation at class I and class II CAP-dependent promoters permits “anti-activation” by negative regulators. Basic features of transcription activation at class I and class II CAP-dependent promoters appear to be generalizable to other activators.

Keywords: catabolite activator protein (CAP); cyclic AMP receptor protein (CRP); RNA polymerase; transcription initiation; transcription activation

Introduction

The Escherichia coli catabolite activator protein (CAP; also known as the cAMP receptor protein, CRP) activates transcription at more than 100 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 by Kolb et al., 1993a; Ebright, 1993; Busby & Ebright, 1997).

CAP has provided a classic model system for structural and mechanistic studies of transcription activation. Thus, CAP was the first transcription activator to have been purified (Zubay et al., 1970; Emmer et al., 1970) and the first transcription activator to have its three-dimensional structure determined (McKay & Steitz, 1981), and transcription activation by CAP has been the subject of extensive biophysical, biochemical, and genetic investigations (Kolb et al., 1993a; Ebright, 1993; Busby & Ebright, 1997).

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 (Ebright, 1993; Busby & Ebright,
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; Gralla & Collado-Vides, 1996), and very substantially simpler than examples of transcription activation in eukaryotes (which require dozens of macromolecular components and DNA sites; Roeder, 1996; Orphanides et al., 1997). Accordingly, it has been possible to develop structural and mechanistic descriptions of transcription activation by CAP that are more nearly complete than descriptions of any other examples of transcription activation.

In this review, we introduce the three macromolecular components required for transcription activation at the simplest CAP-dependent promoters (CAP, RNAP, and promoter DNA), and we present structural and mechanistic descriptions of transcription activation at each of two classes of simple CAP-dependent promoters. In addition, we show that basic principles derived from study of transcription activation at simple CAP-dependent promoters can illuminate understanding of transcription activation at more complex CAP-dependent promoters and at other activator-dependent promoters.

**Macromolecular components**

**CAP**

CAP has a molecular mass of 45 kDa and is a dimer of two identical subunits (Kolb et al., 1993a). Each subunit consists of two domains. The N-terminal domain (residues 1-139) is responsible for dimerization of CAP and for interaction with the allosteric effector cAMP (which binds to CAP and induces a conformational change, resulting in a conformation competent for DNA binding). The C-terminal domain (residues 140-209) is responsible for interaction with DNA, mediating interaction with DNA through a helix-turn-helix DNA-binding motif (for reviews of the helix-turn-helix motif, see Brennan, 1991, 1992). CAP recognizes a 22 bp, 2-fold-symmetric DNA site (consensus sequence 5'-AAATGTGATCTAGATCACATTT-3').

The crystallographic structure of CAP has been determined (McKay & Steitz, 1981), and several crystallographic structures of CAP in complex with DNA have been determined (Figure 1; Schultz et al., 1991; Parkinson et al. 1996a,b; Passner & Steitz, 1997; S. Chen, G. Parkinson, J. Liu, B. Benoff, H. Berman & R.H.E., unpublished results). The CAP-DNA complex is 2-fold symmetric: one CAP subunit interacts with one half of the DNA site, and the other CAP subunit interacts in a 2-fold symmetry-related fashion with the other half of the DNA site. CAP sharply bends DNA in the CAP-DNA complex, bending DNA to an angle of \( \approx 80^\circ \). The orientation of the CAP-induced DNA bend is such that the DNA wraps toward and around the sides of CAP.

**RNAP**

RNAP has a molecular mass of 450 kDa and has subunit composition \( \alpha_2\beta\beta'/\sigma \) (Chamberlin, 1976; Burgess, 1976).

The \( \alpha \) subunit (37 kDa) is responsible for recognition of the UP element (a supplementary promoter element located upstream of the \( -35 \) element in certain promoters; Ross et al., 1993), and for response to a large subset of activators, repressors, elongation factors, and termination factors (Busby & Ebright, 1994; Ebright & Busby, 1995; Hochschild & Dove, 1998; Liu & Hanna, 1995; Liu et al., 1996; Schauer et al., 1996; Kainz & Gourse, 1998). The \( \alpha \) subunit consists of two independently
folded domains (Blatter et al., 1994; Negishi et al., 1995; Busby & Ebright, 1994; Ebright & Busby, 1995). The α subunit N-terminal domain (αNTD; residues 8-235) contains the primary determinant for dimerization of α, the primary determinant for interaction of α with the remainder of RNAP, and a determinant for interaction with activators. The α subunit C-terminal domain (αCTD; residues 249-329) contains a secondary, weak determinant for dimerization of α and determinants for interactions with DNA (including sequence-specific interactions with UP-element DNA and non-specific interactions with non-UP-element DNA), activators, repressors, elongation factors, and termination factors. The linker between αNTD and αCTD is at least 13 amino acid residues in length (≥44 Å if fully extended) and is unstructured and flexible (Blatter et al., 1994; Negishi et al., 1995; Jeon et al., 1997). The long, unstructured, flexible linker allows αCTD to occupy different positions relative to αNTD, and thus relative to the remainder of RNAP, in different transcription complexes (Blatter et al., 1994; Busby & Ebright, 1994; Ebright & Busby, 1995).

The β (151 kDa) and β′ (155 kDa) subunits are responsible for the catalytic activity of RNAP and for response to a subset of activators, repressors, elongation factors, and termination factors (Chamberlin, 1976; Severinov et al., 1994; Miller et al., 1997; Nечаев & Severinov, 1999).

The σ70 subunit (70 kDa) (in this review, we refer only to σ70, the principal σ subunit) is responsible for recognition of the promoter −35 element and −10 element (recognised by σ70 region 4 and σ70 region 2, respectively) and for response to a subset of activators (Busby & Ebright, 1994; Gross et al., 1998).

Low-resolution structures of RNAP and of RNAP core (subunit composition α2ββ′) have been determined using electron microscopy and image reconstruction (Поляков et al., 1995; Darst et al., 1998). Recently, a high-resolution structure of RNAP core has been determined using X-ray crystallography (S. Darst, personal communication). In addition, high-resolution structures of αNTD (Zhang & Darst, 1998), αCTD (Jeon et al., 1995; J. Liu, G. Parkinson, E. Blatter, H. Berman, & R.H.E., unpublished results), and σ70 region 2 (Malhotra et al., 1996) have been determined.

**CAP-dependent promoters**

CAP-dependent promoters can be grouped into three classes (Ushida & Aiba, 1990; Ebright, 1993): (i) class I CAP-dependent promoters require only CAP for transcription activation, and have a single DNA site for CAP located upstream of the DNA site for RNAP. The DNA site for CAP can be located at various distances from the transcription start point, provided that the DNA site for CAP and the DNA site for RNAP are on the same face of the DNA helix. Thus, the DNA site for CAP can be centered near position −93, position −83, position −72, or position −62. The best-characterized class I CAP-dependent promoters are the lac promoter and the artificial promoter CC(−61.5) (Gaston et al., 1990), each of which has a DNA site for CAP centered at position −61.5.

(ii) Class II CAP-dependent promoters require only CAP for transcription activation, and have a single DNA site for CAP overlapping the DNA site for RNAP, apparently replacing the promoter −35 element. The best-characterized class II CAP-dependent promoters are the galP1 promoter and the artificial promoter CC(−41.5) (Gaston et al., 1990), each of which has a DNA site for CAP centered at position −41.5.

(iii) Class III CAP-dependent promoters require multiple activator molecules for full transcription activation, i.e. two or more CAP molecules, or one or more CAP molecule and one or more regulon-specific activator molecule. Examples include the ansB promoter (Scott et al., 1995), the arabAD promoter (Lobell & Schleif, 1991; Zhang & Schleif, 1998), the malk promoter (Richter et al., 1991), and the uhpT promoter (Merkel et al., 1995).

**Transcription activation at class I CAP-dependent promoters**

**CAP determinants**

Transcription activation at the lac promoter requires a determinant consisting of residues 156-164 of CAP, located within the C-terminal domain of CAP, immediately preceding the helix-turn-helix DNA-binding motif of CAP ("activating region 1", A1; Bell et al., 1990; Eschenlauer & Reznikoff, 1991; Zhou et al., 1993a; Niu et al., 1994). Single amino acid substitutions within AR1 reduce or eliminate transcription activation at the lac promoter, but does not affect DNA binding and DNA bending by CAP (Zhou et al., 1993a). Alanine scanning indicates that the side-chain of Thr158 is the most important side-chain for function of AR1 (Niu et al., 1994). AR1 folds as a canonical type I β-turn and forms a prominently exposed surface patch with dimensions of ≈11 Å × ≈14 Å (Figure 1(a)). Experiments with "oriented heterodimers" of CAP having one subunit with a functional AR1 and one subunit with a non-functional AR1 indicate that transcription activation at lac requires a functional AR1 only in the downstream subunit of the CAP dimer (Figure 1(a); Zhou et al., 1993b).

AR1 is essential for transcription activation, not only at lac and other class I CAP-dependent promoters in which the DNA site for CAP is centered near position −62, but also at class I CAP-dependent promoters in which the DNA site for CAP is centered further upstream (e.g. near position −93, position −83, or position −72) (Zhou et al., 1994a). Oriented-heterodimer analysis indicates that, in each case, AR1 is functionally presented in the downstream subunit of the CAP dimer (Zhou et al., 1994b).
RNAP determinants

Transcription activation at the lac promoter requires CTD (Igarashi & Ishihama, 1991). Thus, RNAP reconstituted with truncated \( \alpha \) subunits lacking CTD is defective in CAP-dependent transcription at lac, but not defective in CAP-independent transcription at lacUV5 (a CAP-independent mutant of lac; Beckwith et al., 1972). Experiments with “oriented-alpha” RNAP derivatives having one full-length \( \alpha \) subunit and one truncated \( \alpha \) subunit lacking CTD indicate that only one of the two copies of CTD in RNAP is essential for transcription activation at lac, and that this copy can be, interchangeably, CTD\( ^{1} \) (CTD of the \( \alpha \) subunit that interacts with \( \beta \)') or CTD\( ^{11} \) (CTD of the \( \alpha \) subunit that interacts with \( \beta \)')(Zou et al., 1993; W. Niu & R.H.E., unpublished).

Isolation and characterization of single amino acid substitutions in CTD that result in specific defects in class I CAP-dependent transcription at lac has led to the identification of three critical determinants within CTD, each named by the position at which substitutions result in the most severe defects (Figure 2; Zou et al., 1992; Tang et al., 1994; Murakami et al., 1996; Savery et al., 1998; N. Savery, R. Gourse, R.H.E. & S.B., unpublished results):

(i) Residues in the “265 determinant” (Arg265, Asn268, Asn294, Gly296, Lys298, Ser299, Glu302) form a surface with dimensions \( \approx 14 \, \text{Å} \times \approx 23 \, \text{Å} \). The 265 determinant is required for CTD-DNA interaction (Gaal et al., 1996; Murakami et al., 1996). Accordingly, the 265 determinant is involved in UP-element-dependent transcription as well as in CAP-dependent transcription (Gaal et al., 1996; Murakami et al., 1996).

(ii) Residues in the “261 determinant” (Val257, Asp258, Asp259, Glu261) form a surface with dimensions \( \approx 7 \, \text{Å} \times \approx 16 \, \text{Å} \) that is adjacent to, but distinct from, the 265 determinant. The 261 determinant is not required for CTD-DNA interaction (Tang et al., 1994), but, nevertheless, is important for UP-element-dependent transcription as well as CAP-dependent transcription (W. Ross & R. Gourse, unpublished; H. Chen & R.H.E., unpublished).

(iii) Residues in the “287 determinant” (Thr285, Glu286, Val287, Glu288, Leu289, Gly315, Arg317, Leu318) form a surface with dimensions \( \approx 11 \, \text{Å} \times \approx 22 \, \text{Å} \) that is adjacent to, but distinct from, the 265 determinant, and is on the face of CTD opposite the 261 determinant. The 287 determinant is not required for CTD-DNA interaction and plays no role in UP-element-dependent transcription (Gaal et al., 1996; Savery et al., 1998). Substitutions in the 287 determinant reduce or eliminate CAP-\( \alpha \) cooperativity in experiments assessing formation of CAP-\( \alpha \)-DNA complexes (Savery et al., 1998). Therefore, it is proposed that the 287 determinant is essential for protein-protein interactions between CAP and \( \alpha \) on promoter DNA.

DNA determinants

Hydroxyl radical and DNase I DNA footprinting experiments indicate that formation of the ternary complex of CAP, RNAP, and the lac promoter results in protection, not only of the DNA site for CAP and the core promoter, but also of the DNA segment immediately downstream of the DNA site for CAP (positions \( -50 \) to \( -41 \); Kolb et al., 1993b). Full protection of this DNA segment requires the determinants in CAP and RNAP described above; thus, substitution of AR1 results in a reduction of protection, and removal of CTD results in the complete loss of protection (Kolb et al., 1993b).

Transcription activation at the lac promoter is sensitive to the structural integrity of the DNA segment immediately downstream of the DNA site for CAP (the same DNA segment protected in the DNA footprinting experiments). Thus, gaps in this DNA segment reduce or eliminate transcription activation (Ryu et al., 1994). Transcription activation at the lac promoter also is sensitive to the sequence of this DNA segment. In the wild-type lac promoter, this DNA segment does not correspond to a high-affinity DNA site for CTD and, indeed, appears to contain no specific sequence information (Flatow et al., 1996; Czarniecki et al., 1997). However, replacement of this DNA segment by a high-affinity DNA site for CTD, i.e. an UP-element subsite (consensus sequence, 5’-AAAAAAARNA-3’; Estrem et al., 1999) results in an increase in transcription (Czarniecki et al., 1997; Noel & Reznikoff, 1998; see also Savery et al., 1995; Law et al., 1999). The optimal spacing between the DNA site for CAP and the UP-element subsite appears to be four to five base-pairs (Czarniecki

Figure 2. Structure of CTD showing determinants involved in CAP-dependent transcription (265 determinant, red; 261 determinant, blue; 287 determinant, yellow; Jeon et al., 1995; J. Liu, G. Parkinson, E. Blatter, H. Berman & R.H.E., unpublished results).
Mechanism

Protein-protein photocrosslinking indicates that, in the ternary complex of CAP, RNAP, and the lac promoter, AR1 of CAP is in direct physical proximity to αCTD of RNAP (Chen et al., 1994). Furthermore, protein-DNA photocrosslinking indicates that, in the complex, αCTD is in direct physical proximity to the DNA segment immediately downstream of the DNA site for CAP (N. Naryshkin, A. Revyakin, Y. Kim & R.H.E., unpublished results). Together with the above-described results defining critical determinants of CAP, RNAP, and promoter DNA, the photocrosslinking results lead to the proposal that transcription activation at the lac promoter involves a direct protein-protein interaction between AR1 of the downstream subunit of the CAP dimer and one of the two copies of αCTD of RNAP (interchangeably αCTD⁴ or αCTD⁵) that facilitates binding of that copy of αCTD to the DNA segment immediately downstream of CAP (i.e. the DNA segment between the downstream subunit of the CAP dimer and σ⁷⁰ region 4 bound at the promoter –35 element) (Figure 3(a)).

The 287 determinant of αCTD is proposed to mediate the protein-protein interaction with AR1 of CAP, and the 265 determinant of αCTD is proposed to mediate the protein-DNA interaction with the DNA segment immediately downstream of CAP (Figure 3(a)). The role of the 261 determinant of CAP, and the 265 determinant of αCTD is proposed to permit establishment at such promoters of the same local CAP-αCTD and αCTD-DNA interactions as at the lac promoter, despite the difference in position of the DNA site for CAP (cf. Figures 3(a) and (b)). At such promoters, as at the lac promoter, CAP-αCTD interaction is proposed to increase the affinity of RNAP for promoter DNA, resulting in an increase in Kₘ and, thus, an increase in transcription. (In the special case of the malT promoter, which has a DNA site for CAP centered at position –70.5 and an inhibitory high-affinity DNA site for αCTD centered near position –47, CAP-αCTD interaction serves to prevent αCTD from interacting with the inhibitory high-affinity DNA site, and thereby to prevent formation of a non-productive RNAP-promoter complex deficient in promoter escape (Tagami & Aiba, 1998, 1999).)

At class I CAP-dependent promoters, all CAP-RNAP and RNAP-DNA interactions essential for transcription activation are made downstream of the DNA site for CAP, and thus downstream of the locus of CAP-induced DNA bending (Figure 3; Zhou et al., 1993b, 1994b; W. Niu, N. Naryshkin, A. Revyakin, Y. Kim & R.H.E., unpublished results). Therefore, it appears unlikely that CAP-induced DNA bending plays an essential role in transcription activation at class I CAP-dependent promoters. Consistent with this inference, mutants of CAP that result in a decrease in CAP-induced DNA bending are not defective in transcription activation at lac, neither in vivo nor in vitro (A. Kapanidis & R.H.E., unpublished results).
Figure 3. Transcription activation at class I CAP-dependent promoters. ζCTD, ζNTD, β, β’, and σ denote, respectively, the RNAP ζ subunit C-terminal domain, the RNAP ζ subunit N-terminal domain, and the RNAP β, β’, and σ20 subunits. ζCTD is an independently folded module and is connected to ζNTD, and thus to the remainder of RNAP, through a unstructured, flexible linker; alternative positioning of ζCTD is facilitated by the linker and by bending of the intervening DNA (Blatter et al., 1994; Busby & Ebright, 1994; Ebright & Busby, 1995). For simplicity, DNA is drawn straight; in point of fact, both CAP and RNAP bend DNA (Schultz et al., 1991; Parkinson et al., 1996a,b; Rees et al., 1993; Meyer-Almes et al., 1994; Rivetti et al., 1999). (a) Ternary complex of CAP, RNAP, and a class I CAP-dependent promoter having the DNA site for CAP centered near position –62, e.g. lac, CC(−61.5). Transcription activation involves direct protein-protein interaction between AR1 of the downstream subunit of CAP (open, dashed circle) and the 287 determinant of one copy of ζCTD. The AR1-ζCTD interaction facilitates binding of ζCTD, through its 265 determinant, to the DNA segment immediately downstream of CAP and, possibly, through its 261 determinant, to σ20 region 4 bound at the −35 element. The location of the second, non-contacted copy of ζCTD has not been determined definitively; therefore, the second copy of ζCTD is drawn arbitrarily behind the first. Available evidence suggests that the second copy of ζCTD may interact, through its 265 determinant, with the DNA segment upstream of the DNA site for CAP (Kolb et al., 1993b; N.N., A.R., Y.K. & R.H.E., unpublished results). (b) Ternary complex of CAP, RNAP, and a class I CAP-dependent promoter having the DNA site for CAP centered near position –103, position –93, position –83, or position –72, e.g. malT, CC(−71.5). Transcription activation involves the same AR1-ζCTD and ζCTD-DNA interactions as in (a). Available evidence suggests that the second, non-contacted copy of ζCTD may interact, through its 265 determinant, with the DNA segment immediately upstream of the −35 element and, possibly, through its 261 determinant, with σ20 region 4 bound at the −35 element (Eichenberger et al., 1996; Law et al., 1999; N.S. & S.B., unpublished results). (Adapted from Blatter et al., 1994; Zhou et al., 1994a,b; additions based on Savery et al., 1998; N. Savery, W. Ross, R. Gourse, R.H.E. & S.B., unpublished results; and H.C., H.T. & R.H.E., unpublished results.)

determinant consisting of residues His19, His21, Glu21, and Lys101 of CAP, located in the N-terminal, cAMP-binding domain of CAP ("activating region 2", AR2; Niu et al., 1996). AR2 was identified by isolation of mutants of CAP defective in transcription activation at class II CAP-dependent promoters, but not defective in transcription activation at class I CAP-dependent promoters, DNA binding, and DNA bending (Niu et al., 1996). In the structure of the CAP-DNA complex, the residues that comprise AR2 form a prominently exposed surface with dimensions of \( \approx 8 \AA \times \approx 20 \AA \). AR2 carries a net positive charge of +2, and mutational studies indicate that net positive charge is critical for AR2 function. Oriented-heterodimer analysis indicates that AR2 is functionally presented by the downstream subunit of the CAP dimer (Williams et al., 1996; Niu et al., 1996).

In the structure of the CAP-DNA complex, the two functional determinants critical for transcription activation at class II CAP-dependent promoters, i.e. AR1 in the upstream subunit of the CAP dimer and AR2 in the downstream subunit of the CAP dimer, map to the same face of the CAP dimer, but are separated by nearly the full length of this face (Figure 1(b)).

**RNAP determinants**

Class II CAP-dependent transcription requires two sets of determinants in the RNAP ζ subunit: (i) Class II CAP-dependent transcription, like class I CAP-dependent transcription, requires ζCTD (in this case, the 265 and 287 determinants of ζCTD (Figure 2; Savery et al., 1998))†. Experiments with oriented-ζ RNAP derivatives having one full-length ζ subunit and one truncated ζ subunit lacking ζCTD indicate that only one of the two copies of ζCTD in RNAP is required for class II CAP-dependent transcription, and that this copy can be

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† Two reports in the literature incorrectly conclude that ζCTD plays no positive role in class II CAP-dependent transcription (Igarashi et al., 1991; West et al., 1993). The incorrect conclusions in these reports are attributable to errors in normalization of specific activities of wild-type and mutant RNAP preparations (D. West & S.B., unpublished results). RNAP derivatives lacking ζCTD and properly normalized with respect to specific activity exhibit defects in class II CAP-dependent transcription (D. West & S.B., unpublished results; W. Niu & R.H.E., unpublished results).
either αCTD\(^1\) (αCTD of the α subunit that interacts with β) or, less favorably, αCTD\(^{II}\) (αCTD of the α subunit that interacts with β') (W.N. & R.H.E., unpublished results).

(ii) Class II CAP-dependent transcription requires a class-II-specific determinant within αNTD (Niu et al., 1996). This determinant, which consists of residues 162-165 within αNTD, was defined by the isolation of single amino acid substitution mutants defective in class II CAP-dependent transcription, but not defective in class I CAP-dependent transcription or CAP-independent transcription (Niu et al., 1996). In the structure of αNTD, the residues that comprise this determinant are located within a prominently accessible surface loop (Zhang & Darst, 1998). All four residues that comprise this determinant are negatively charged, and net negative charge appears to be critical for function of the determinant (Niu et al., 1996). Preliminary experiments with oriented-α RNAP derivatives carrying one wild-type and one mutant α subunit indicate that this determinant is functionally presented in only one of the two α subunits, i.e. α', the α subunit that interacts with β (W. Niu & R.H.E., unpublished results).

**DNA determinants**

Hydroxyl radical and DNase I DNA footprinting experiments indicate that formation of the ternary complex of CAP, RNAP, and a class II CAP-dependent promoter results in protection, not only of the DNA site for CAP and the core promoter, but also of the DNA segment immediately upstream of the DNA site for CAP (Attey et al., 1994; Belyaeva et al., 1996, 1998). Full protection of this DNA segment requires the integrity of both AR1 of CAP and αCTD. At the best-characterized class II CAP-dependent promoter, CC(−41.5), there is no specific DNA sequence determinant in this DNA segment. Nevertheless, replacement of this DNA segment by a high-affinity DNA site for αCTD, such as an UP-element or an UP-element subsite, results in an increase in transcription (Lloyd et al., 1998). In such cases, the optimal spacing between the DNA site for CAP and the UP-element or UP-element subsite appears to be four base-pairs (Lloyd et al., 1998; G. Lloyd, W. Niu, R.H.E. & S.B., unpublished results).

At a class II CAP-dependent promoter, the presence of a consensus DNA site for CAP overlapping the −35 element precludes the presence of a consensus −35 element. DNA affinity cleaving experiments with RNAP derivatives having EDTA:Fe incorporated within σ\(^{−20}\) region 4 indicate that σ\(^{−20}\) region 4 interacts with the non-consensus −35 element of a class II CAP-dependent promoter in a manner largely similar to that in which it interacts with the consensus −35 element of a consensus promoter (J. Bown, A. Kolb, A. Ishihama & S.B., unpublished results). Consistent with this finding, transcription initiation is sensitive to DNA sequence within the −35-element region of a class II CAP-dependent promoter (Rhodius et al., 1997).

**Mechanism**

Protein-protein photocrosslinking indicates that, in the ternary complex of CAP, RNAP, and a class II CAP-dependent promoter, AR1 of CAP is in direct physical proximity to αCTD of RNAP (Y. Chen, W. Niu & R.H.E., unpublished results), and AR2 of CAP is in direct physical proximity to the αNTD of RNAP (Niu et al., 1996). DNA affinity cleaving with RNAP derivatives having EDTA:Fe incorporated within αCTD indicates that, in the complex, αCTD is in direct physical proximity to the DNA segment immediately upstream of the DNA site for CAP (Murakami et al., 1997). Together with the above-described results defining critical determinants of CAP, RNAP, and promoter DNA, the photocrosslinking and affinity cleaving results lead to the proposal that CAP activates transcription at class II promoters through two distinct sets of interactions (Figure 4):

(i) AR1 of the upstream subunit of the CAP dimer is proposed to interact with one of the two copies of αCTD of RNAP (either αCTD\(^1\) or αCTD\(^{II}\), but preferentially αCTD\(^1\)), facilitating binding of that copy of αCTD to the DNA segment immediately upstream of CAP. The interaction with AR1 is proposed to involve the 287 determinant of αCTD, and the interaction with DNA is proposed to involve the 265 determinant of αCTD.

(ii) AR2 of the downstream subunit of CAP is proposed to interact with αNTD\(^1\), interacting with residues 162-165 within αNTD\(^1\). The two sets of interactions have separate and distinct mechanistic consequences for transcription activation (Niu et al., 1996; Rhodius et al., 1997):

(i) The interaction between AR1 and αCTD increases the binding constant, \(K_p\), for the formation of the RNAP-promoter complex and has no effect on the rate constant, \(k_p\), for subsequent isomerization of the closed complex to the open complex (Niu et al., 1996; Rhodius et al., 1997). The increase in \(K_p\) arises from two sources. First, and most obvious, the AR1-αCTD interaction directly increases the affinity of RNAP for promoter DNA (W.N. & R.H.E., unpublished results; D. West & S.B., unpublished results). Second, the AR1-αCTD interaction compensates the energetic cost of displacing αCTD from its preferred location on promoter DNA and positioning αCTD at a less preferred location on promoter DNA (“anti-inhibition”; Busby & Ebright, 1997), an energetic cost imposed by the fact that αCTD prefers to interact with DNA in the −42 region (N. Naryshkin, A. Revyakin, Y. Kim, H. Chen, H. Tang & R.H.E., unpublished) and the fact that, at class II CAP-dependent promoters, CAP binds to DNA in the −42 region, necessitating displacement of αCTD.

(ii) The interaction between AR2 and αNTD, in contrast to the interaction between AR1 and αCTD, functions at a step subsequent to the
initial binding of RNAP to promoter DNA (Niu et al., 1996; Rhodius et al., 1997). Thus, this interaction does not affect the binding constant, $K_B$, for formation of the RNAP-promoter closed complex, but, rather, increases the rate constant, $k_B$, for isomerization of closed complex to open complex. The mechanism by which the AR2-$\alpha$NTD interaction facilitates isomerization is not known. Based on the structure of the CAP-DNA complex and the position of the DNA site for CAP at class II promoters, the AR2-$\alpha$NTD interaction is expected to take place more than 30 Å from the DNA and more than 70 Å from the RNAP active site (Niu et al., 1996). In principle, two mechanisms are possible: the AR2-$\alpha$NTD interaction may trigger an allosteric change between an inactive RNAP conformation and an active RNAP conformation, or the AR2-$\alpha$NTD interaction may selectively stabilize the transition state between closed complex and open complex (Niu et al., 1996).

Transcription activation at class II CAP-dependent promoters thus provides a paradigm for understanding how a single activator molecule can make multiple interactions with the transcription machinery, with each interaction being responsible for a specific mechanistic consequence. Such multiple interactions are likely to be a common feature of transcription activation.

Transcription activation at class II CAP-dependent promoters involves interactions that take place upstream of the DNA site for CAP, and thus upstream of the locus of CAP-induced DNA bending (Figure 4). Therefore, it appears likely that CAP-induced DNA bending plays a role in transcription activation at class II CAP-dependent promoters, i.e. facilitation of upstream CAP-$\alpha$CTD and $\alpha$CTD-DNA interactions. Consistent with this inference, substitutions in CAP that result in a decrease in CAP-induced bending result in a measurable (albeit modest) defect in transcription activation at class II CAP-dependent promoters (A. Kapanidis & R.H.E., unpublished).

A third, non-native CAP-RNAP interaction at class II CAP-dependent promoters

AR1 and AR2 can be supplemented by a third, non-native activating region (residues 52-58; “activating region 3”, AR3; Bell et al., 1990; Williams et al., 1991; West et al., 1993; Niu et al., 1996). AR3 is created by substitution of Lys52 by a neutral or negatively charged residue. Substitution of Lys52 substantially increases transcription activation at class II CAP-dependent promoters, but not class I CAP-dependent promoters, and at least partly suppresses effects of substitutions in AR1, AR2, or both (Bell et al., 1990; Williams et al., 1991; West et al., 1993; V. Rhodius & S.B, unpublished results). In addition, substitution of Lys52 substantially strengthens the interaction in solution between CAP-DNA binary complexes and RNAP (Niu et al., 1996). We suggest that substitution of Lys52 exerts these effects by creating a non-native energetically favorable interaction with RNAP.

Alanine scanning of a CAP derivative having a functional AR3 (i.e. a CAP derivative with a substitution of Lys52) indicates that Glu58 is essential for the function of AR3 (Williams et al., 1991; V. Rhodius & S.B., unpublished results). In the structure of the wild-type CAP-DNA complex, Lys52 and Glu58 form a salt bridge. We suggest that substitution of Lys52 “unmasks” the negative charge of Glu58 and thereby creates a non-native, energetically favorable, electrostatic interaction with RNAP. Oriented-heterodimer analysis indicates that AR3, when functional, is presented by the downstream subunit of the CAP dimer (Figure 1(b); Williams et al., 1996).

Several lines of evidence indicate that AR3, when functional, interacts with $\sigma^{70}$ region 4, specifically with residues 590-600, which immediately follow the helix-turn-helix motif responsible for recognition of the promoter $\sigma^{35}$ element. First, substitution of Lys593, Lys597, or Arg599 of $\sigma^{70}$ reduces or eliminates AR3-dependent transcription activation at class II CAP-dependent promoters (Lonetto et al., 1998). Second, site-specific protein-protein photocrosslinking indicates that AR3 is in
direct physical proximity to $\sigma^{70}$ in the ternary complex of CAP, RNAP, and a class II CAP-dependent promoter (Jin et al., 1995). Third, model building suggests that AR3 of the downstream subunit of CAP is in direct physical proximity to residues 590-600 of $\sigma^{70}$ in the ternary complex (Busby & Ebright, 1997; Lonetto et al., 1998). Fourth, AR3, when active, carries a net negative charge and therefore is electrostatically complementary to residues 590-600 of $\sigma^{70}$, which carry a net positive charge.

The AR3-$\sigma^{70}$ interaction has no effect on the binding constant, $K_b$, for formation of the RNAP-promoter closed complex, and affects only the rate constant, $k_r$, for isomerization of closed complex to the open complex (V. Rhodius & S.B., unpublished results). Interestingly, $\lambda$C I, another activator that interacts with residues 590-600 of $\sigma^{70}$ (Li et al., 1994), and that is electrostatically complementary to residues 590-600 of $\sigma^{70}$ (Bushman et al., 1989), likewise affects only $k_r$ (Hawley & McClure, 1982).

**Transcription activation at class III CAP-dependent promoters**

**Synergistic transcription activation by multiple CAP dimers**

At some CAP-dependent promoters, two or more CAP dimers synergistically activate transcription. Such promoters have diverse architectures, with different distances between the two DNA sites for CAP, and different distances between the DNA sites for CAP and the DNA site for RNAP. Remarkably, despite the apparently complex, diverse architectures of these promoters, transcription activation at such promoters is relatively simple, involving straightforward, additive combinations of the elementary class I and class II mechanisms.

Thus, a CAP dimer centered near position $-103$ or position $-93$ can synergistically activate transcription with a CAP dimer centered near position $-62$ (Joung et al., 1993; Law et al., 1999; Langdon & Hochschild, 1999). In such cases, each CAP dimer functions through a class I mechanism, with AR1 of the downstream subunit of each CAP dimer interacting with one copy of $\alpha$CTD (Figure 5(a)).

Similarly, a CAP dimer centered near position $-103$, position $-93$, or position $-83$ can synergistically activate transcription with a CAP dimer centered near position $-42$ (Busby et al., 1994; Murakami et al., 1997; Belyaeva et al., 1998). In such cases, the upstream CAP dimer functions by a class I mechanism, with AR1 of the downstream subunit interacting with one copy of $\alpha$CTD; and the downstream CAP dimer functions by a class II mechanism, with AR1, AR2, and, if present, AR3, interacting with, respectively, the other copy of $\alpha$CTD, $\alpha$NTD, and $\sigma^{70}$ region 4 (Figure 5(b)).

**Synergistic transcription activation by CAP and other activators**

At many CAP-dependent promoters, CAP synergistically activates transcription with one or more other activators.

At some promoters where CAP synergistically activates transcription with a second, different activator, the mechanisms of transcription activation are similar to the mechanisms described in the preceding section.

Thus, a CAP dimer centered near position $-103$ or position $-93$ can synergistically activate transcription with a second, different activator able to interact with $\alpha$CTD, $\alpha$NTD, and/or $\sigma^{70}$ (e.g. $\lambda$C I or FNR centered near position $-42$; Joung et al., 1994; Scott et al., 1995). In such cases, CAP functions by a class I mechanism, with AR1 of the downstream subunit of CAP interacting with one copy of $\alpha$CTD, and the second activator interacts with the other copy of $\alpha$CTD, $\alpha$NTD, and/or $\sigma^{70}$ (cf. Figure 5(b)).

Similarly, a CAP dimer centered near position $-42$ can synergistically activate transcription with a second, different activator able to interact with $\alpha$CTD (e.g. FNR centered near position $-103$, position $-93$, or position $-83$; Busby et al., 1994; Savery et al., 1996). In such cases, CAP functions through a class II mechanism, with AR1, AR2, and, if present, AR3, interacting with, respectively, one copy of $\alpha$CTD, $\alpha$NTD, and $\sigma^{70}$; and the second activator interacts with the other copy of $\alpha$CTD (cf. Figure 5(b)).

In each of these cases, synergistic transcription activation results from the fact that CAP and the second, different activator make independent contacts with different surfaces of RNAP (and possibly also affect different steps in transcription initiation). Importantly, these mechanisms for synergistic transcription activation do not require direct interaction between CAP and the second activator, and thus it is possible for CAP to synergistically activate transcription through these mechanisms with a broad range of unrelated activators.

At other promoters where CAP functions together with a second, different activator, the mechanisms of transcription activation are more complex. Thus, at some promoters, CAP functions, at least in part, through direct protein-protein interaction with a second activator that facilitates interactions between the second activator and DNA, through CAP-induced DNA bending that facilitates interactions between a second activator and RNAP, and/or through CAP-induced DNA bending that disrupts inhibitory interactions (Lobell & Schleif, 1991; Richet et al., 1991; Forsman et al., 1992; Perez-Martin & Espinosa, 1993; Merkel et al., 1995). At such promoters, the AR1-$\alpha$CTD, AR2-$\alpha$NTD, and AR3-$\sigma^{70}$ interactions critical for transcription activation at class I and class II CAP-dependent promoters play little or no role.
Anti-activation

Anti-activation by blocking CAP or DNA determinants: CytR

CytR uses an “anti-activation” mechanism to inhibit transcription initiation at a subset of CAP-dependent promoters involved in pyrimidine metabolism, most notably the deoP2, udp, nupG, and cdd promoters (Mollegaard et al., 1993; Valentin-Hansen et al., 1996). Each of these promoters has a DNA site for CAP centered near position −94 and a second DNA site for CAP centered near position −42 (as in the class III CAP-dependent promoters in Figure 5(b)). In the absence of the allosteric effector cytidine, CytR inhibits transcription initiation at these promoters by making protein-protein interactions with the two CAP dimers bound to promoter DNA and protein-DNA interactions with the DNA segment between the two CAP dimers (Figure 5(c)). The CytR-CAP and CytR-DNA interactions completely block transcription activation by CAP, by sterically blocking the functional AR1 of each CAP dimer and by sterically preventing αCTD from interacting with the DNA segment adjacent to each CAP dimer (cf. Figures 5(b) and (c)).

The determinant of CAP involved in CytR-CAP interaction has been identified by isolation of single amino acid substitutions that render CAP insensitive to anti-activation by CytR, but that do not interfere with transcription activation, DNA binding, or DNA bending by CAP (Søgaard-Andersen et al., 1991; Meibom et al., 1999). The determinant consists of residues Glu12, Trp13, His17, Leu105, Val108, and Pro110 in the N-terminal, cAMP-binding domain of CAP. Oriented-heterodimer analysis indicates that, for each of the two CAP dimers, the determinant is functionally presented in the CAP subunit proximal to the DNA site for CytR (K. Meibom, B. Kallipolitis, P. Valentin-Hansen & R.H.E., unpublished results).

The determinant of CytR that mediates CytR-CAP interaction also has been identified, and a detailed model for the structural organization of the (CAP)₂-CytR-DNA anti-activation complex has been proposed (Kallipolitis et al., 1997).

Anti-activation by blocking RNAP determinants: bacteriophage T4 ADP-ribosylation

Interference with transcription activation by CAP can also be accomplished by blocking essential determinants on RNAP. During infection of E. coli by bacteriophage T4, the T4 alt and mod gene products ADP-ribosylate Arg265 of RNAP α subunit (Goff, 1984), the most critical residue of the 265 determinant of αCTD (Gaal et al., 1996;

Implications

The mechanisms summarized here for transcription activation by CAP can be generalized to other bacterial activators.

In particular, these mechanisms apply with full force to FNR, a distant sequence and structural homolog of CAP (Guest et al., 1996). FNR activates transcription at promoters organized precisely analogously to class I, class II, and class III CAP-dependent promoters (Wing et al., 1995; Scott et al., 1995). FNR contains functional counterparts of AR1 and AR3, and FNR activates transcription through AR1-ςCTD and AR3-ς70 interactions that, in all significant respects, are equivalent to the interactions made by CAP and summarized in Figures 3 and 4 (Williams et al., 1997; Li et al., 1998; Lonetto et al., 1998).

A large subset of bacterial activators unrelated in sequence and structure to CAP also appears to activate transcription by interacting with promoter DNA upstream of the −35 element and making protein-protein interactions with ςCTD that, in all significant respects, are equivalent to those made by CAP at class I CAP-dependent promoters and summarized in Figure 3 (Busby & Ebright, 1994; Ebright & Busby, 1995; Rhodius & Busby, 1998). Another large subset of bacterial activators appears to function by interacting with promoter DNA in the −35 element region and making concurrent protein-protein interactions with ςCTD, ςNTD, and/or ς70 analogous to those made by CAP at class II CAP-dependent promoters and summarized in Figure 4 (Busby & Ebright, 1994; Rhodius & Busby, 1998).

Prospect

Transcription activation at class I, class II, and the simplest class III CAP-dependent promoters should be amenable to a complete structural and mechanistic description. Priorities for future work include elucidation of the structures of the AR1-ςCTD, AR2-ςNTD, and AR3-ς70 interfaces; determination of when CAP-RNAP interactions are first made on the pathway from free promoter to RNAP-promoter closed complex, to RNAP-promoter open complex; and determination of when, and how, these interactions are broken in promoter escape. Methods to address these priorities are in place. Progress should be rapid.

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