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Transcription Activation by Catabolite Activator Protein (CAP)

Steve Busby[1] and Richard H. Ebright[2]*

[1]*School of Biosciences, The University of Birmingham Birmingham B15 2TT, UK*

[2]*Howard Hughes Medical Institute, Waksman Institute and, Department of Chemistry Rutgers University, New Brunswick, NJ 08855, USA*

**Corresponding author*

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 a 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,

Abbreviations used: CAP, catabolite activator protein; CRP, cAMP receptor protein; RNAP, RNA polymerase; αNTD, α subunit N-terminal domain; αCTD, α subunit C-terminal domain; AR1, activating region 1.

E-mail address of the corresponding author: ebright@mbcl.rutgers.edu

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1997). 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 ≈80°. The orientation of the CAP-induced DNA bend is such that the DNA wraps toward and around the sides of CAP.

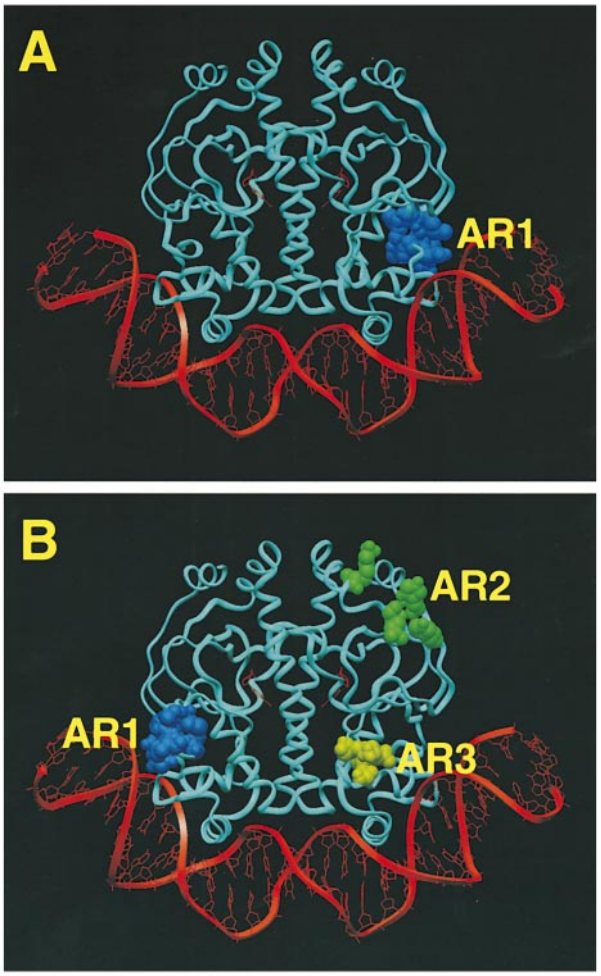

Figure 1. Structure of the CAP-DNA complex showing determinants of CAP involved in transcription activation (CAP, light blue; DNA and cAMP bound to CAP, red; Schultz *et al*., 1991; Parkinson *et al*., 1996a). (a) Determinant of CAP for transcription activation at class I CAP-dependent promoters (AR1 of downstream subunit, dark blue). (b) Determinants of CAP for transcription activation at class II CAP-dependent promoters (AR1 of upstream subunit, dark blue; AR2 of downstream subunit, green; AR3 of downstream subunit, yellow).

RNAP

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

The α 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 α 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 ($\geqslant$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; Nechaev & Severinov, 1999).

The σ^{70} subunit (70 kDa) (in this review, we refer only to σ^{70}, the principal σ subunit) is responsible for recognition of the promoter −35 element and −10 element (recognised by σ^{70} region 4 and σ^{70} 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 $\alpha_2\beta\beta'$) have been determined using electron microscopy and image reconstruction (Polyakov *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 σ^{70} 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 (Richet *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'', AR1; 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 αCTD (Igarashi & Ishihama, 1991). Thus, RNAP reconstituted with truncated α 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 α subunit and one truncated α subunit lacking CTD indicate that only one of the two copies of αCTD in RNAP is essential for transcription activation at *lac*, and that this copy can be, interchangeably, αCTD^{I} (αCTD of the α subunit that interacts with β) or αCTD^{II} (αCTD of the α subunit that interacts with β′)(Zou *et al.*, 1993; W. Niu & R.H.E., unpublished).

Isolation and characterization of single amino acid substitutions in αCTD that result in specific defects in class I CAP-dependent transcription at *lac* has led to the identification of three critical determinants within αCTD, 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 ≈14 Å × ≈ 23 Å. The 265 determinant is required for αCTD-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).

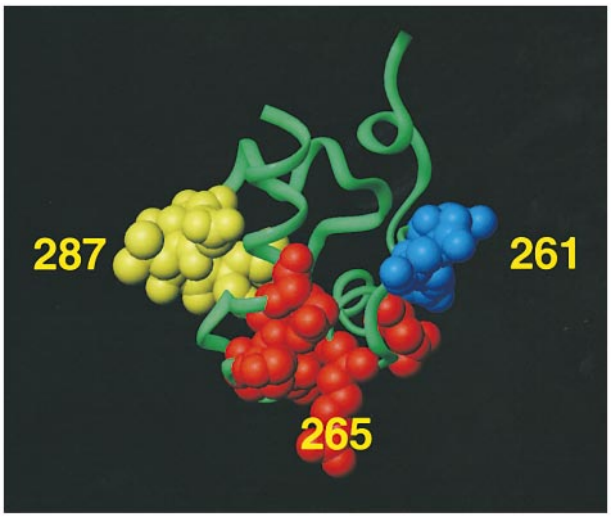

Figure 2. Structure of αCTD showing determinants involved in CAP-dependent transcription (265 determinant, red; 261 determinant, blue; 287 determinant, yellow; Jeon *et al.*, 1995; J. Liu, G. Parkinson, E. Blatter, H. Berman & R.H.E., unpublished results).

(ii) Residues in the ''261 determinant'' (Val257, Asp258, Asp259, Glu261) form a surface with dimensions ≈7 Å × ≈ 16 Å that is adjacent to, but distinct from, the 265 determinant. The 261 determinant is not required for αCTD-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 ≈11 Å × ≈22 Å that is adjacent to, but distinct from, the 265 determinant, and is on the face of αCTD opposite the 261 determinant. The 287 determinant is not required for αCTD-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-α cooperativity in experiments assessing formation of CAP-α-DNA complexes (Savery *et al.*, 1998). Therefore, it is proposed that the 287 determinant is essential for protein-protein interactions between CAP and α 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 αCTD 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 αCTD 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 αCTD, i.e. an UP-element subsite (consensus sequence, 5′-AAAAAARNA-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

et al., 1997; see also Savery *et al.*, 1995; Law *et al.*, 1999).

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 αCTD^{I} or αCTD^{II}) 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 σ^{70} 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 αCTD is not completely understood. Preliminary results suggest that the 261 determinant mediates a protein-protein interaction with σ^{70} region 4 bound at the promoter −35 element (Figure 3(a); W. Ross & R. Gourse, unpublished results; H. Chen, H. Tang & R.H.E., unpublished results).

The interactions between CAP and RNAP at the *lac* promoter increase the affinity of RNAP for promoter DNA, resulting in an increase in the binding constant, K_B, for formation of the RNAP-promoter closed complex and, thus, an increase in transcription initiation (Malan *et al.*, 1984; see also Ren *et al.*, 1988; Straney *et al.*, 1989; Kolb *et al.*, 1993b; Heyduk *et al.*, 1993). All available data are consistent with the proposal that CAP activates transcription at *lac* solely by helping ''recruit'' RNAP to promoter DNA. In particular, removal of CAP from the pre-formed CAP-RNAP-promoter open complex (by addition of high concentrations of heparin) has no negative effects on subsequent steps in transcription initiation, elongation, and termination (Tagami & Aiba, 1995).

At other class I CAP-dependent promoters in which the DNA site for CAP is located near position −62, the mechanism of transcription activation is proposed to be identical with that at the *lac* promoter (Zhou *et al.*, 1994a,b).

At class I CAP-dependent promoters in which the DNA site for CAP is located further upstream (e.g. near position −93, position 83, or position −72), CAP is proposed to activate transcription by making a protein-protein interaction between AR1 of the downstream subunit of the CAP dimer and αCTD analogous to that at the *lac* promoter, causing αCTD to make a protein-DNA interaction with the DNA segment immediately downstream of CAP analogous to that at the *lac* promoter (Figure 3(b); Zhou *et al.*, 1994a,b; Blatter *et al.*, 1994). The unstructured, flexible linker between the αCTD and the remainder of RNAP (Blatter *et al.*, 1994; Negishi *et al.*, 1995; Jeon *et al.*, 1997), possibly together with bending of the intervening DNA, 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 K_B 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).

Transcription activation at class II CAP-dependent promoters

CAP determinants

Transcription activation at class II CAP-dependent promoters requires two distinct determinants in CAP (Figure 1(b)):

(i) Transcription activation at class II CAP-dependent promoters, like transcription activation at class I CAP-dependent promoters, requires AR1 (Bell *et al.*, 1990; Williams *et al.*, 1991; West *et al.*, 1993; Zhou *et al.*, 1994a). Oriented-heterodimer analysis indicates that, at class II CAP-dependent promoters, AR1 is functionally presented by the upstream subunit of the CAP dimer (Zhou *et al.*, 1994b).

(ii) Transcription activation at class II CAP-dependent promoters requires a class II-specific

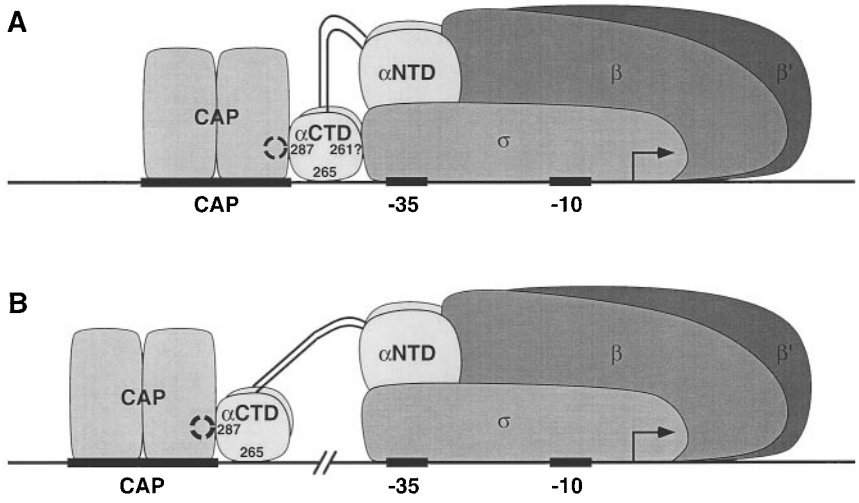

Figure 3. Transcription activation at class I CAP-dependent promoters. αCTD, αNTD, β, β′, and σ denote, respectively, the RNAP α subunit C-terminal domain, the RNAP α subunit N-terminal domain, and the RNAP β, β′, and σ^{70} subunits. αCTD is an independently folded module and is connected to αNTD, and thus to the remainder of RNAP, through a unstructured, flexible linker; alternative positioning of αCTD 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 αCTD. The AR1-αCTD interaction facilitates binding of αCTD, through its 265 determinant, to the DNA segment immediately downstream of CAP and, possibly, through its 261 determinant, to σ^{70} region 4 bound at the −35 element. The location of the second, non-contacted copy of αCTD has not been determined definitively; therefore, the second copy of αCTD is drawn arbitrarily behind the first. Available evidence suggests that the second copy of αCTD 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-αCTD and αCTD-DNA interactions as in (a). Available evidence suggests that the second, non-contacted copy of αCTD may interact, through its 265 determinant, with the DNA segment immediately upstream of the −35 element and, possibly, through its 261 determinant, with σ^{70} 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 ≈8 Å × ≈20 Å. 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 α subunit: (i) Class II CAP-dependent transcription, like class I CAP-dependent transcription, requires αCTD (in this case, the 265 and 287 determinants of αCTD (Figure 2; Savery *et al.*, 1998))†. Experiments with oriented-α RNAP derivatives having one full-length α subunit and one truncated α subunit lacking αCTD indicate that only one of the two copies of αCTD in RNAP is required for class II CAP-dependent transcription, and that this copy can be

† Two reports in the literature incorrectly conclude that αCTD 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 αCTD 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^{I} (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^{I}, 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^{70} region 4 indicate that σ^{70} 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^{I} or αCTD^{II}, but preferentially αCTD^{I}), 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^{I}, interacting with residues 162-165 within αNTD^{I}.

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_B, for the formation of the RNAP-promoter closed complex and has no effect on the rate constant, k_f, for subsequent isomerization of the closed complex to the open complex (Niu *et al*., 1996; Rhodius *et al*., 1997). The increase in K_B 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

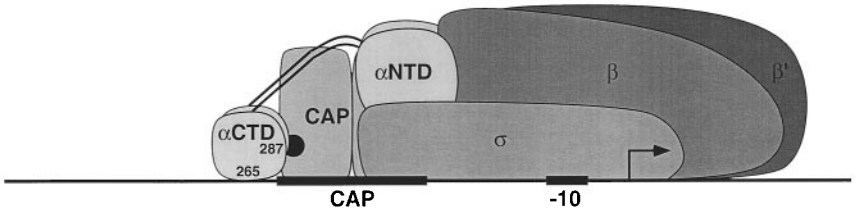

Figure 4. Transcription activation at class II CAP-dependent promoters, e.g. *galP1*, *CC(−41.5)*. Transcription activation at class II CAP-dependent promoters involves two CAP-RNAP interactions: (i) interaction between AR1 of the upstream subunit of CAP (filled circle) and the 287 determinant of one copy of αCTD, an interaction that facilitates binding of αCTD, through its 265 determinant to the DNA segment immediately upstream of CAP; and (ii) interaction between AR2 of the downstream subunit of CAP (not visible in this orientation; located directly beneath the N in αNTD) and residues 162-165 of one copy of αNTD. The location of the second, non-contacted copy of αCTD has not been determined definitively; therefore, the second copy of αCTD is drawn arbitrarily behind the first. Available evidence suggests that the second copy of αCTD may interact, through its 265 determinant, with the DNA segment upstream of the first copy of αCTD (Belyaeva *et al.*, 1996, 1998; Murakami *et al.*, 1997; Lloyd *et al.*, 1998). Adapted from Niu *et al.* (1996).

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_f, for isomerization of closed complex to open complex. The mechanism by which the AR2-αNTD 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-αNTD 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-αNTD interaction may trigger an allosteric change between an inactive RNAP conformation and an active RNAP conformation, or the AR2-αNTD 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-αCTD and αCTD-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 σ^{70} region 4, specifically with residues 590-600, which immediately follow the helix-turn-helix motif responsible for recognition of the promoter −35 element. First, substitution of Lys593, Lys597, or Arg599 of σ^{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 σ^{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 σ^{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 σ^{70}, which carry a net positive charge.

The AR3-σ^{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_f, for isomerization of closed complex to the open complex (V. Rhodius & S.B., unpublished results). Interestingly, λcI, another activator that interacts with residues 590-600 of σ^{70} (Li *et al.*, 1994), and that is electrostatically complementary to residues 590-600 of σ^{70} (Bushman *et al.*, 1989), likewise affects only k_f (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 αCTD (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 αCTD; 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 αCTD, αNTD, and σ^{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 αCTD, αNTD, and/or σ^{70} (e.g. λcI 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 αCTD, and the second activator interacts with the other copy of αCTD, αNTD, and/or σ^{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 αCTD (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 αCTD, αNTD, and σ^{70}; and the second activator interacts with the other copy of αCTD (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-αCTD, AR2-αNTD, and AR3-σ^{70} interactions critical for transcription activation at class I and class II CAP-dependent promoters play little or no role.

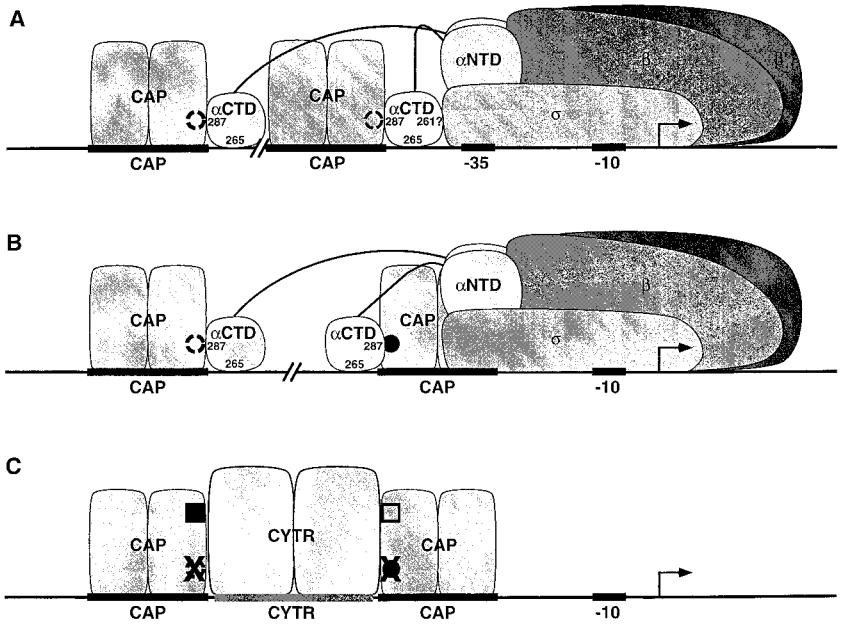

Figure 5. Transcription activation and anti-activation at class III CAP-dependent promoters. (a) Quaternary complex of two CAP dimers, RNAP, and a class III CAP-dependent promoter having one DNA site for CAP centered near position −103 or position −93, and a second DNA site for CAP centered near position −62. Each CAP dimer functions through a class I mechanism, with AR1 of the downstream subunit of each CAP dimer (open, dashed circle) interacting with one copy of αCTD (cf. Figure 3). (Adapted from Langdon & Hochschild, 1999). (b) Quaternary complex of two CAP dimers, RNAP, and a class III CAP-dependent promoter having one DNA site for CAP centered near position −103, position −93, or position −83, and a second DNA site for CAP centered near position −42. The upstream CAP dimer functions by a class I mechanism, with AR1 of the downstream subunit (open, dashed circle) interacting with one copy of αCTD (cf. Figure 3(b)); the downstream CAP dimer functions by a class II mechanism, with AR1 (filled circle), AR2 (not visible in this orientation; located directly beneath the N in αNTD), and, if present, AR3, interacting with, respectively, the other copy of αCTD, αNTD, and σ^{70} region 4 (cf. Figure 4). (Adapted from Murakami *et al*., 1997; Belyaeva *et al*., 1998.) (c) Ternary complex of CytR, two CAP dimers, and a CytR-regulated class III CAP-dependent promoter (''anti-activation complex''). CytR makes protein-protein interactions with the two CAP dimers, interacting with residues 12, 13, 17, 105, 108 and 110 of the CytR-proximal subunit of each CAP dimer (squares), and protein-DNA interactions with the DNA segment between the two CAP dimers. The CytR-CAP and CytR-DNA interactions block transcription activation by CAP (anti-activate) by blocking the functional AR1 of each CAP dimer and by preventing interaction of αCTD with the DNA segment adjacent to each CAP dimer (cf. (b)). (Adapted from Valentin-Hansen *et al*., 1996; Kallipolitis *et al*., 1997.)

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;

Murakami *et al.*, 1996; Savery *et al.*, 1998). ADP-ribosylation of Arg265 blocks αCTD-DNA interaction and thus selectively abolishes CAP-dependent transcription and UP-element-dependent transcription, without affecting CAP-independent, UP-element-independent transcription (K. Severinov, W. Ross, H. Tang, L. Snyder, A. Goldfarb, R. Gourse & R.H.E., unpublished results).

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-αCTD and AR3-σ^{70} 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 αCTD 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 αCTD, αNTD, and/or σ^{70} 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-αCTD, AR2-αNTD, and AR3-σ^{70} 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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References

Attey, A., Belyaeva, T., Savery, N., Hoggett, J., Fujita, N., Ishihama, A. & Busby, S. (1994). Interactions between the cyclic AMP receptor protein and the alpha subunit of RNA polymerase at the *E. coli galP1* promoter. *Nucl. Acids Res.* **22**, 4375-4380.

Beckwith, J., Grodzicker, T. & Arditti, R. (1972). Evidence for two sites in the *lac* promoter region. *J. Mol. Biol.* **69**, 155-160.

Bell, A., Gaston, K., Williams, R., Chapman, K., Kolb, A., Buc, H., Minchin, S., Williams, J. & Busby, S. (1990). Mutations that affect the ability of the *Escherichia coli* cyclic AMP receptor protein to activate transcription. *Nucl. Acids Res.* **17**, 3865-3874.

Belyaeva, T., Bown, J., Fujita, N., Ishihama, A. & Busby, S. (1996). Location of the C-terminal domain of the RNA polymerase alpha subunit in different open complexes at the *E. coli* galactose operon regulatory region. *Nucl. Acids Res.* **24**, 2243-2251.

Belyaeva, T., Rhodius, V., Webster, C. & Busby, S. (1998). Transcription activation at promoters carrying tandem DNA sites for the *Escherichia coli* cyclic AMP receptor protein: organization of the RNA polymerase α subunits. *J. Mol. Biol.* **277**, 789-804.

Blatter, E., Ross, W., Tang, H., Gourse, R. & Ebright, R. (1994). Domain organization of RNA polymerase alpha subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell,* **78**, 889-896.

Brennan, R. (1991). Interactions of the helix-turn-helix binding domain. *Curr. Opin. Struct. Biol.* **1**, 80-88.

Brennan, R. (1992). DNA recognition by the helix-turn-helix motif. *Curr. Opin. Struct. Biol.* **2**, 100-108.

Burgess, R. (1976). Purification and physical properties of *E. coli* RNA polymerase. In *RNA Polymerase* (Losick, R. & Chamberlin, M., eds), pp. 69-100, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Busby, S. & Ebright, R. (1994). Promoter structure, promoter recognition, and transcription activation in prokaryotes. *Cell,* **79**, 743-746.

Busby, S. & Ebright, R. (1997). Transcription activation at class II CAP-dependent promoters. *Mol. Microbiol.* **23**, 853-859.

Busby, S., West, D., Lawes, M., Webster, C., Ishihama, A. & Kolb, A. (1994). Transcription activation by the *E. coli* cyclic AMP receptor protein: receptors bound in tandem at promoters can interact synergistically. *J. Mol. Biol.* **241**, 341-352.

Bushman, F., Shang, C. & Ptashne, M. (1989). A single glutamic acid residue plays a key role in the transcriptional activation function of lambda repressor. *Cell,* **58**, 1163-1171.

Chamberlin, M. (1976). RNA polymerase - an overview. In *RNA Polymerase* (Losick, R. & Chamberlin,

M., eds), pp. 17-67, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Chen, Y., Ebright, Y. & Ebright, R. (1994). Identification of the target of a transcription activator protein by protein-protein photocrosslinking. *Science,* **265**, 90-92.

Czarniecki, D., Noel, R. & Reznikoff, W. (1997). The −45 region of the *Escherichia coli lac* promoter: CAP-dependent and CAP-independent transcription. *J. Bacteriol.* **179**, 423-429.

Darst, S., Polyakov, A., Richter, C. & Zhang, G. (1998). Insights into *Escherichia coli* RNA polymerase structure from a combination of X-ray and electron crystallography. *J. Struct. Biol.* **124**, 115-122.

Ebright, R. (1993). Transcription activation at class I CAP-dependent promoters. *Mol. Microbiol.* **8**, 797-802.

Ebright, R. & Busby, S. (1995). *Escherichia coli* RNA polymerase alpha subunit: structure and function. *Curr. Opin. Genet. Dev.* **5**, 197-200.

Eichenberger, P., Dethiollaz, S., Fujita, N., Ishihama, A. & Geiselmann, J. (1996). Influence of the location of the cAMP receptor protein binding site on the geometry of a transcriptional activation complex in *Escherichia coli*. *Biochemistry,* **35**, 15302-15312.

Emmer, M., de Crombrugghe, B., Pastan, I. & Perlman, R. (1970). Cyclic AMP receptor protein of *E. coli*: its role in the synthesis of inducible enzymes. *Proc. Natl Acad. Sci. USA,* **66**, 480-487.

Eschenlauer, A. & Reznikoff, W. (1991). *Escherichia coli* catabolite gene activator protein mutants defective in positive control of lac operon transcription. *J. Bacteriol.* **173**, 5024-5029.

Estrem, S., Ross, W., Gaal, ?., Chen, Z. W. S., Niu, W. & Ebright, R. (1999). Bacterial promoter architecture: subsite structure of UP elements and interactions with the carboxyl-terminal domain of RNA polymerase α subunit. *Genes Dev.* **13**, 2134-2147.

Flatow, U., Rajendrakumar, G. & Garges, S. (1996). Analysis of the spacer DNA between the cyclic AMP receptor protein site and the *lac* promoter. *J. Bacteriol.* **178**, 2436-2439.

Forsman, K., Sonden, B., Goransson, M. & Uhlin, B. E. (1992). Antirepression function in *Escherichia coli* for the cAMP-cAMP receptor protein transcriptional activator. *Proc. Natl Acad. Sci. USA,* **89**, 9880-9884.

Gaal, T., Ross, W., Blatter, E., Tang, H., Jia, X., Krishnan, V., Assa-Munt, N., Ebright, R. & Gourse, R. (1996). DNA-binding determinants of the alpha subunit of RNA polymerase: novel DNA-binding domain architecture. *Genes Dev.* **10**, 16-26.

Gaston, K., Bell, A., Kolb, A., Buc, H. & Busby, S. (1990). Stringent spacing requirements for transcription activation by CRP. *Cell,* **62**, 733-740.

Goff, C. (1984). Coliphage-induced ADP-ribosylation of *Escherichia coli* RNA polymerase. *Methods Enzymol.* **106**, 418-429.

Gralla, J. & Collado-Vides, J. (1996). Organization and function of transcription regulatory elements. In *Escherichia coli* and *Salmonella* (Neidhardt, F., ed.), pp. 1232-1245, ASM Press, Washington, DC.

Gross, C., Chan, C., Dombroski, A., Gruber, T., Sharp, M., Tupy, J. & Young, B. (1998). The functional and regulatory roles of sigma factors in transcription. *Cold Spring Harbor Symp. Quant. Biol.* **63**, 141-155.

Guest, J., Green, J., Irvine, A. & Spiro, S. (1996). The FNR modulon and FNR-regulated gene expression. In *Regulation of Gene Expression in* E. coli (Lin, E. C. C. & Lynch, A. S., eds), pp. 317-342, R.G. Landes, New York.

Hawley, D. & McClure, W. (1982). Mechanism of activation of transcription initiation from the lambda P_{RM} promoter. *J. Mol. Biol.* **157**, 493-525.

Heyduk, T., Lee, J., Ebright, Y., Blatter, E., Zhou, Y. & Ebright, R. (1993). CAP-RNA polymerase interaction in solution in the absence of promoter DNA. *Nature,* **364**, 548-549.

Hochschild, A. & Dove, S. (1998). Protein-protein contacts that activate and repress prokaryotic transcription. *Cell,* **92**, 597-600.

Igarashi, K. & Ishihama, A. (1991). Bipartite functional map of the *E. coli* RNA polymerase α subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. *Cell,* **65**, 1015-1022.

Igarashi, K., Hanamura, A., Makino, K., Aiba, H., Aiba, H., Mizuno, T., Nakata, A. & Ishihama, A. (1991). Functional map of the alpha subunit of *Escherichia coli* RNA polymerase: two modes of transcription activation by positive factors. *Proc. Natl Acad. Sci. USA,* **88**, 8958-8962.

Jeon, Y., Negishi, T., Shirakawa, M., Yamazaki, T., Fujita, N., Ishihama, A. & Kyogoku, M. (1995). Solution structure of the activator contact domain of the RNA polymerase α subunit. *Science,* **270**, 1495-1497.

Jeon, Y. H., Yamazaki, T., Otomo, T., Ishihama, A. & Kyogoku, Y. (1997). Flexible linker in the RNA polymerase alpha subunit facilitates the independent motion of the C-terminal activator contact domain. *J. Mol. Biol.* **267**, 953-962.

Jin, R., Sharif, K. & Krakow, J. (1995). Evidence for contact between the cyclic AMP receptor protein and the σ^{70} subunit of *Escherichia coli* RNA polymerase. *J. Biol. Chem.* **270**, 19213-19216.

Joung, J., Le, L. & Hochschild, A. (1993). Synergistic activation of transcription by *Escherichia coli* cAMP receptor protein. *Proc. Natl Acad. Sci. USA,* **90**, 3083-3087.

Joung, J., Koepp, D. & Hochschild, A. (1994). Synergistic activation of transcription by bacteriophage λ cI protein and *E. coli* cAMP receptor protein. *Science,* **265**, 1863-1866.

Kainz, M. & Gourse, R. (1998). The C-terminal domain of the alpha subunit of *Escherichia coli* RNA polymerase is required for Rho-dependent transcription termination. *J. Mol. Biol.* **284**, 1379-1390.

Kallipolitis, B., Norregaard-Madsen, M. & Valentin-Hansen, P. (1997). Protein-protein communication: structural model of the repression complex formed by CytR and the global regulator CRP. *Cell,* **89**, 1101-1109.

Kolb, A., Busby, S., Buc, H., Garges, S. & Adhya, S. (1993a). Transcriptional regulation by cyclic AMP and its receptor protein. *Annu. Rev. Biochem.* **62**, 749-795.

Kolb, A., Igarashi, K., Ishihama, A., Lavigne, M., Buckle, M. & Buc, H. (1993b). *E. coli* RNA polymerase, deleted in the C-terminal part of its α-subunit, interacts differently with the cAMP-CRP complex at the *lacP1* and at the *galP1* promoter. *Nucl. Acids Res.* **21**, 319-326.

Langdon, R. & Hochschild, A. (1999). A novel genetic method for dissecting the mechanism of transcriptional activator synergy by identical activators. *Proc. Natl Acad. Sci. USA,* in the press.

Law, E., Savery, N. & Busby, S. (1999). Interactions between the *Escherichia coli* cAMP receptor protein

and the C-terminal domain of the α subunit of RNA polymerase at class I promoters. *Biochem. J.* **337**, 415-423.

Li, B., Wing, H., Lee, D., Wu, H.-C. & Busby, S. (1998). Transcription activation by *Escherichia coli* FNR protein: similarities to, and differences from, the CRP paradigm. *Nucl. Acids Res.* **26**, 2075-2081.

Li, M., Moyle, H. & Susskind, M. (1994). Target of the transcriptional activation function of phage λ cI protein. *Science,* **263**, 75-77.

Liu, K. & Hanna, M. M. (1995). NusA interferes with interactions between the nascent RNA and the C-terminal domain of the alpha subunit of RNA polymerase in *Escherichia coli* transcription complexes. *Proc. Natl Acad. Sci. USA,* **92**, 5012-5016.

Liu, K., Zhang, Y., Severinov, K., Das, A. & Hanna, M. (1996). Role of *Escherichia coli* RNA polymerase alpha subunit in modulation of pausing, termination and anti-termination by the transcription elongation factor NusA. *EMBO J.* **15**, 150-161.

Lloyd, G., Busby, S. & Savery, N. (1998). Spacing requirements for interactions between the C-terminal domain of the α subunit of *Escherichia coli* RNA polymerase and the cyclic AMP receptor protein. *Biochem. J.* **330**, 413-420.

Lobell, R. & Schleif, R. (1991). AraC DNA looping: orientation and distance-dependent loop breaking by the cyclic AMP receptor protein. *J. Mol. Biol.* **218**, 45-54.

Lonetto, M., Rhodius, V., Lamberg, K., Kiley, P., Busby, S. & Gross, C. (1998). Identification of a contact site for different transcription activators in Region 4 of the *Escherichia coli* RNA polymerase σ^{70} subunit. *J. Mol. Biol.* **284**, 1353-1365.

Malan, T., Kolb, A., Buc, H. & McClure, W. (1984). Mechanism of CRP-cAMP activation of the *lac* operon: transcription initiation activation of the P1 promoter. *J. Mol. Biol.* **180**, 881-909.

Malhotra, A., Severinova, E. & Darst, S. (1996). Crystal structure of a σ^{70} subunit fragment from *E. coli* RNA polymerase. *Cell,* **87**, 127-136.

McKay, D. & Steitz, T. (1981). Structure of catabolite gene activator protein at 2.9 Angstroms resolution suggests binding to left-handed *B*-DNA. *Nature,* **290**, 744-749.

Meibom, K., Søgaard-Andersen, L., Mironov, A. & Valentin-Hansen, P. (1999). Dissection of a surface-exposed portion of the cAMP-CRP complex that mediates transcription activation and repression. *Mol. Microbiol.* **32**, 497-504.

Merkel, T., Dahl, J., Ebright, R. & Kadner, R. (1995). Transcription activation at the *Escherichia coli uhpT* promoter by the catabolite gene activator protein. *J. Bacteriol.* **177**, 1712-1718.

Meyer-Almes, F.-J., Heumann, H. & Porschke, D. (1994). The structure of the RNA polymerase-promoter complex: DNA-bending angle by quantitative electrooptics. *J. Mol. Biol.* **236**, 1-6.

Miller, A. D. W., Ebright, R. & Rothman-Denes, L. (1997). RNA polymerase beta′ subunit: a target for DNA binding-independent activation. *Science,* **275**, 1655-1657.

Mollegaard, N. E., Rasmussen, P. B., Valentin-Hansen, P. & Nielsen, P. (1993). Characterization of promoter recognition complexes formed by CRP and CytR for repression and by CRP and RNA polymerase for activation of transcription on the *Escherichia coli deo*P2 promoter. *J. Biol. Chem.* **268**, 17471-17477.

Murakami, K., Fujita, N. & Ishihama, A. (1996). Transcription factor recognition surface on the RNA polymerase α subunit is involved in contact with the DNA enhancer element. *EMBO J.* **15**, 4358-4367.

Murakami, K., Owens, J., Belyaeva, T., Meares, C., Busby, S. & Ishihama, A. (1997). Positioning of two alpha subunit carboxy-terminal domains of RNA polymerase along promoter DNA by two transcription factors. *Proc. Natl Acad. Sci. USA,* **94**, 11274-11278.

Nechaev, S. & Severinov, K. (1999). Inhibition of *Escherichia coli* RNA polymerase by bacteriophage T7 gene 2 protein. *J. Mol. Biol.* **289**, 815-826.

Negishi, T., Fujita, N. & Ishihama, A. (1995). Structural map of the alpha subunit of *Escherichia coli* RNA polymerase: structural domains identified by proteolytic cleavage. *J. Mol. Biol.* **248**, 723-728.

Niu, W., Zhou, Y., Dong, Q., Ebright, Y. & Ebright, R. (1994). Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). I. Saturation and alanine scanning mutagenesis. *J. Mol. Biol.* **243**, 595-602.

Niu, W., Kim, Y., Tau, G., Heyduk, T. & Ebright, R. (1996). Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. *Cell,* **87**, 1123-1134.

Noel, R. & Reznikoff, W. (1998). CAP, the −45 region, and RNA polymerase: three partners in transcription initiation at *lacP1* in *Escherichia coli*. *J. Mol. Biol.* **282**, 495-504.

Orphanides, G., Lagrange, T. & Reinberg, D. (1996). The general transcription factors of RNA polymerase II. *Genes Dev.* **10**, 2657-2683.

Parkinson, G., Wilson, C., Gunasekera, A., Ebright, Y., Ebright, R. & Berman, H. (1996a). Structure of the CAP-DNA complex at 2.5 Å resolution. *J. Mol. Biol.* **260**, 395-408.

Parkinson, G., Gunasekera, A., Vojtechovsky, J., Zhang, X., Kunkel, T. & Ebright, R. (1996b). Aromatic hydrogen bond in sequence-specific protein-DNA interaction. *Nature Struct. Biol.* **3**, 837-841.

Passner, J. & Steitz, T. (1998). The structure of a CAP-DNA complex having two cAMP molecules bound to each monomer. *Proc. Natl Acad. Sci. USA,* **94**, 2843-2847.

Perez-Martin, J. & Espinosa, M. (1993). Protein-induced bending as a transcriptional switch. *Science,* **260**, 805-807.

Polyakov, A., Severinova, E. & Darst, S. (1995). Three-dimensional structure of *E. coli* core RNA polymerase: promoter binding and elongation conformations of the enzyme. *Cell,* **83**, 365-373.

Rees, W., Keller, R., Vesenka, J., Yang, G. & Bustamante, C. (1993). Evidence of DNA bending in transcription complexes imaged by scanning force microscopy. *Science,* **260**, 1646-1649.

Ren, Y. L., Garges, S., Adhya, S. & Krakow, J. (1988). Cooperative DNA binding of heterologous proteins: evidence for contact between the cyclic AMP receptor protein and RNA polymerase. *Proc. Natl Acad. Sci. USA,* **85**, 4138-4142.

Rhodius, V. & Busby, S. (1998). Positive activation of gene expression. *Curr. Opin. Microbiol.* **1**, 152-159.

Rhodius, V., West, D., Webster, C., Busby, S. & Savery, N. (1997). Transcription activation at class II CRP-dependent promoters: the role of different activating regions. *Nucl. Acids Res.* **25**, 326-333.

Richet, E., Vidal-Ingigliardi, D. & Raibaud, O. (1991). A new mechanism for coactivation of transcription

initiation: repositioning of an activator triggered by the binding of a second activator. *Cell,* **66**, 1185-1195.

Rivetti, C., Guthold, M. & Bustamante, C. (1999). Wrapping of DNA around the *E. coli* RNA polymerase open promoter complex. *EMBO J.* **18**, 4464-4475.

Roeder, R. (1996). The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem. Sci.* **21**, 327-335.

Ross, W., Gosink, K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K. & Gourse, R. (1993). A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. *Science,* **262**, 1407-1413.

Ryu, S., Garges, S. & Adhya, S. (1994). An arcane role of DNA in transcription activation. *Proc. Natl Acad. Sci. USA,* **91**, 8582-8586.

Savery, N., Rhodius, V., Wing, H. & Busby, S. (1995). Transcription activation at *Escherichia coli* promoters dependent on the cyclic AMP receptor protein: effects of binding sequences for the RNA polymerase α subunit. *Biochem. J.* **309**, 77-83.

Savery, N., Belyaeva, T., Wing, H. & Busby, S. (1996). Regulation of promoters by two transcription activators: evidence for a ''simultaneous touching'' model. *Biochem. Soc. Trans.* **24**, 351-353.

Savery, N., Lloyd, G., Kainz, M., Gaal, T., Ross, W., Ebright, R., Gourse, R. & Busby, S. (1998). Transcription activation at class II CRP-dependent promoters: identification of determinants in the C-terminal domain of the RNA polymerase α subunit. *EMBO J.* **17**, 3439-3447.

Schauer, A., Cheng, S-W., Zheng, C., St Pierre, L., Alessi, D., Hidayetoglu, D., Costantino, N., Court, D. & Friedman, D. (1996). The alpha subunit of RNA polymerase and transcription antitermination. *Mol. Microbiol.* **21**, 839-851.

Schultz, S., Shields, G. & Steitz, T. (1991). Crystal structure of a CAP-DNA complex: the DNA is bent by 90 degrees. *Science,* **253**, 1001-1007.

Scott, S., Busby, S. & Beacham, I. (1995). Transcriptional coactivation at the *ansB* promoters: involvement of the activating regions of CRP and FNR when bound in tandem. *Mol. Microbiol.* **18**, 521-532.

Severinov, K., Kashlev, M., Severinova, E., Bass, I., McWilliams, K., Kutter, E., Nikiforov, V., Snyder, L. & Goldfarb, A. (1994). A non-essential domain of *Escherichia coli* RNA polymerase required for the action of the termination factor Alc. *J. Biol. Chem.* **269**, 14254-14259.

Søgaard-Andersen, L., Mironov, A., Pedersen, H., Sukhodelets, V. & Valentin-Hansen, P. (1991). Single amino acid substitutions in the cAMP receptor protein specifically abolish regulation by the CytR repressor in *Escherichia coli*. *Proc. Natl Acad. Sci. USA,* **88**, 4921-4925.

Straney, D., Straney, S. & Crothers, D. (1989). Synergy between *Escherichia coli* CAP protein and RNA polymerase in the lac promoter open complex. *J. Mol. Biol.* **206**, 41-57.

Tagami, H. & Aiba, H. (1995). Role of CRP in transcription activation at the *Escherichia coli lac* promoter: CRP is dispensable after the formation of open complex. *Nucl. Acids Res.* **23**, 599-605.

Tagami, H. & Aiba, H. (1998). A common role of CRP in transcription activation: CRP acts transiently to stimulate events leading to open complex formation at a diverse set of promoters. *EMBO J.* **17**, 1759-1767.

Tagami, H. & Aiba, H. (1999). An inactive open complex mediated by an UP element at *Escherichia coli* promoters. *Proc. Natl Acad. Sci. USA,* **96**, 7202-7207.

Tang, H., Severinov, K., Goldfarb, A., Fenyo, D., Chait, B. & Ebright, R. (1994). Location, structure, and function of the target of a transcriptional activator protein. *Genes Dev.* **8**, 3058-3067.

Ushida, C. & Aiba, H. (1990). Helical phase dependent action of CRP: effect of the distance between the CRP site and the −35 region on promoter activity. *Nucl. Acids Res.* **18**, 6325-6330.

Valentin-Hansen, P., Søgaard-Andersen, L. & Pedersen, H. (1996). A flexible partnership: the CytR anti-activator and the cAMP-CRP activator protein, comrades in transcription control. *Mol. Microbiol.* **20**, 461-466.

West, D., Williams, R., Rhodius, V., Bell, A., Sharma, N., Zou, C., Fujita, N., Ishihama, A. & Busby, S. (1993). Interactions between the *Escherichia coli* cyclic AMP receptor protein and RNA polymerase at class II promoters. *Mol. Microbiol.* **10**, 789-797.

Williams, R., Bell, A., Sims, G. & Busby, S. (1991). The role of two surface exposed loops in transcription activation by the *Escherichia coli* CRP and FNR proteins. *Nucl. Acids Res.* **19**, 6705-6712.

Williams, R., Rhodius, V., Bell, A., Kolb, A. & Busby, S. (1996). Orientation of functional activating regions in the *E. coli* CRP protein during transcription activation at class II promoters. *Nucl. Acids Res.* **24**, 1112-1118.

Williams, S., Savery, N., Busby, S. & Wing, H. (1997). Transcription activation at class I FNR-dependent promoters: identification of the activating surface of FNR and the corresponding contact site in the C-terminal domain of the RNA polymerase α subunit. *Nucl. Acids Res.* **25**, 4028-4034.

Wing, H., Williams, S. & Busby, S. (1995). Spacing requirements for transcription activation by *Escherichia coli* FNR protein. *J. Bacteriol.* **177**, 6704-6710.

Zhang, G. & Darst, S. (1998). Structure of the *Escherichia coli* RNA polymerase alpha subunit amino-terminal domain. *Science,* **281**, 262-266.

Zhang, X. & Schleif, R. (1998). Catabolite gene activator protein mutations affecting activity of the *araBAD* promoter. *J. Bacteriol.* **180**, 195-200.

Zhou, Y., Zhang, X. & Ebright, R. (1993a). Identification of the activating region of CAP: isolation and characterization of mutants of CAP specifically defective in transcription activation. *Proc. Natl Acad. Sci. USA,* **90**, 6081-6085.

Zhou, Y., Busby, S. & Ebright, R. (1993b). Identification of the functional subunit of a dimeric transcription activator protein by use of ''oriented heterodimers''. *Cell,* **73**, 375-379.

Zhou, Y., Merkel, T. & Ebright, R. (1994a). Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). II. Role at class I and class II CAP-dependent promoters. *J. Mol. Biol.* **243**, 603-610.

Zhou, Y., Pendergrast, P., Bell, A., Williams, R., Busby, S. & Ebright, R. (1994b). The functional subunit of a dimeric transcription activator protein depends on promoter architecture. *EMBO J.* **13**, 4549-4557.

Zou, C., Fujita, K., Igarashi, K. & Ishihama, A. (1992). Mapping the cAMP receptor protein contact site on the alpha subunit of *Escherichia coli* RNA polymerase. *Mol. Microbiol.* **6**, 2599-2605.

Zou, C., Fujita, K. & Ishihama, A. (1993). Asymmetric arrangement of two alpha subunits within *Escherichia coli* RNA polymerase. Involvement of one alpha subunit in contact with cAMP receptor protein. *J. Mol. Biol.* **11**, 1283-1288.

Zubay, G., Schwartz, D. & Beckwith, J. (1970). Mechanism of activation of catabolite-sensitive genes: a positive control system. *Proc. Natl Acad. Sci. USA,* **66**, 104-11.