Determinants of the C-Terminal Domain of the *Escherichia coli* RNA Polymerase α Subunit Important for Transcription at Class I Cyclic AMP Receptor Protein-Dependent Promoters

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Alanine scanning of the *Escherichia coli* RNA polymerase α subunit C-terminal domain (α CTD) was used to identify amino acid side chains important for class I cyclic AMP receptor protein (CRP)-dependent transcription. Key residues were investigated further in vivo and in vitro. Substitutions in three regions of α CTD affected class I CRP-dependent transcription from the *CC*(−61.5) promoter and/or the *lacP1* promoter. These regions are (i) the 287 determinant, previously shown to contact CRP during class II CRP-dependent transcription; (ii) the 265 determinant, previously shown to be important for α CTD-DNA interactions, including those required for class II CRP-dependent transcription; and (iii) the 261 determinant. We conclude that CRP contacts the same target in α CTD, the 287 determinant, at class I and class II CRP-dependent promoters. We also conclude that the relative contributions of individual residues within the 265 determinant depend on promoter sequence, and we discuss explanations for effects of substitutions in the 261 determinant.

The α subunits of *Escherichia coli* RNA polymerase holoenzyme (RNAP) play a key role in transcription initiation and activation (reviewed in references 5 and 11). Each α subunit consists of two independently folded domains connected by a flexible linker (4, 12, 13). The N-terminal domain (α NTD) is critical for the assembly of the core RNAP complex (5). The C-terminal domain (α CTD) binds to A/T-rich sequence elements (UP elements) at many promoters (11, 22) and is also a target for transcription activators, with many activators interacting directly with α CTD and recruiting it, and consequently the rest of RNAP, to target promoter DNA (5). The structure of α CTD has been determined by nuclear magnetic resonance spectroscopy (8, 12). The aim of this study was to identify the contact target in α CTD for the transcription activator cyclic AMP (cAMP) receptor protein (CRP; also referred to as catabolite activator protein).

Transcription activation by CRP provides an important model system for understanding mechanisms of bacterial transcriptional regulation (reviewed in reference 6). CRP is a homodimer that binds to DNA in the presence of cAMP. Simple CRP-dependent promoters can be grouped into two classes, depending on the location of the DNA binding site for CRP. At class I CRP-dependent promoters, CRP binds upstream of RNAP, at sites centered near position −61, −71, −82, or −92 upstream from the transcription start site. The best-characterized class I CRP-dependent promoters are *lacP1* and a semisynthetic derivative of the *melR* promoter, *CC*(−61.5) (9), each of which contains a CRP-binding site centered at position

−61.5. At class II CRP-dependent promoters, the CRP-binding site overlaps the binding site for RNAP. The best-characterized class II CRP-dependent promoters are *galP1* and a semisynthetic derivative of the *melR* promoter, *CC*(−41.5) (9), each of which contains a CRP-binding site centered at position −41.5.

At both class I and class II CRP-dependent promoters, CRP interacts with α CTD, facilitating the binding of α CTD to the DNA segment adjacent to the CRP binding site. CRP- α CTD interaction is mediated by a surface-exposed loop comprising residues 156 to 164 of CRP (designated activating region 1 [AR1]). At class I promoters, AR1 is functionally presented by the downstream subunit of the CRP dimer and interacts with α CTD located downstream of CRP, whereas at class II promoters, AR1 is functionally presented by the upstream subunit of the CRP dimer and interacts with α CTD located upstream of CRP. Substitutions within AR1 disrupt CRP- α CTD interactions at both class I and class II promoters, but quantitative effects of alanine substitutions for individual residues differ from promoter to promoter (30).

In previous work, we used random mutagenesis and alanine scanning to identify amino acid side chains of α CTD required for CRP-dependent transcription activation at a class II promoter, *CC*(−41.5) (23). We identified two determinants that play a key role. The first determinant, termed the 287 determinant, included residues T285, E286, V287, E288, and R317. Substitutions in the 287 determinant did not affect sequence-specific α CTD-DNA interactions but did reduce cooperativity of DNA binding by α subunits and CRP (23). The 287 determinant of α CTD therefore was proposed to be the surface directly contacted by AR1 of CRP during transcription activation at class II promoters. The second determinant, termed the

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Reference(s)
Strains		
M182 Δ crp	Δ crp derivative of M182 (<i>E. coli</i> Δ lac K-12 strain)	7
RLG4650	M182 carrying λ prophage with <i>CC</i> (-61.5)::lacZ fusion	This work
RLG4651	M182 carrying λ prophage with <i>lacP1</i> ::lacZ fusion	This work
WAM105	<i>rpoA341</i> derivative of MJF1	26
WAM106	<i>rpoA</i> ⁺ derivative of MJF1	26
WAM140	Derivative of WAM106 with <i>rpoA</i> EA261 mutant	This work
WAM144	Derivative of WAM106 with <i>rpoA</i> VA287 mutant	This work
Plasmids		
pSR	pBR322 derivative containing transcription terminator	16
pSR/ <i>CC</i> (-61.5)	pSR derivative carrying <i>CC</i> (-61.5) promoter fragment (-101 to +35)	This work
pSR/ <i>lacP1</i>	pSR derivative carrying <i>lacP1</i> promoter fragment (-140 to +63)	This work
pSR/ <i>lacUV5</i>	pSR derivative carrying <i>lacUV5</i> promoter fragment (-59 to +37)	23
pSR/ <i>lacUV5</i> (-140/63)	pSR derivative carrying <i>lacUV5</i> promoter fragment (-140 to +63)	This work
pREII α and derivatives	Plasmid carrying <i>rpoA</i> encoding RNAP α subunit and derivatives carrying alanine substitutions at positions 273–329, except 302	4, 8, 14, 25, 28
pHTf1 α and derivatives	Plasmid carrying <i>rpoA</i> encoding RNAP α subunit and derivatives carrying alanine substitutions at positions 255–271 and 302	8, 25
pDCRP	pBR322 derivative carrying <i>crp</i>	3
pDCRP HL159	Derivative of pDCRP encoding CRP HL159	3
pDU9	Derivative of pDCRP with <i>crp</i> gene deleted	3
pRW50	Broad-host-range <i>lac</i> expression vector	17
pRW50/ <i>CC</i> (-61.5)	pRW50 derivative carrying <i>CC</i> (-61.5) promoter fragment (-101 to +266)	27
pRW50/ <i>lacP1</i>	pRW50 derivative carrying <i>lacP1</i> promoter fragment (-140 to +63)	This work
pRW50/ <i>lacUV5</i>	pRW50 derivative carrying <i>lacUV5</i> promoter fragment (-59 to +37)	This work

265 determinant, included R265 and other amino acids previously shown to be required for binding of α CTD to UP elements (8, 11, 20). The 265 determinant was proposed to mediate α CTD-DNA interactions at class II promoters.

In this work, we systematically tested the effects of alanine substitutions throughout the α CTD on CRP-dependent transcription at the class I CRP-dependent promoter *CC*(-61.5). In addition, we evaluated the roles of key residues of α CTD in transcription at *CC*(-61.5) and at a second class I CRP-dependent promoter, *lacP1*, in vivo using strains lacking wild-type α subunits and in vitro using RNAP derivatives reconstituted with α subunits carrying alanine substitutions. We discuss our results in the light of previous reports.

MATERIALS AND METHODS

Strains, plasmids, and promoters. Bacterial strains and plasmids used in this study are listed in Table 1. Promoter fragments were cloned as *EcoRI-HindIII* fragments. Strains RLG4650 and RLG4651 were constructed by the method of Simons et al. (24), as described previously (23). Strains WAM140 and WAM144 were constructed by exploiting the observation that the *rpoA341* mutation in strain WAM105 results in a Cym⁻ Mel⁻ phenotype (26). WAM140 and WAM144 were obtained as Cym⁺ Mel⁺ revertants resulting from recombination with plasmid-borne 3' segments of *rpoA* encoding the EA261 and VA287 substitutions, respectively. After curing the *rpoA* plasmid, the chromosomal *rpoA* gene was checked by DNA sequencing.

Strain RLG4650 and plasmid pSR/*CC*(-61.5) contain positions -101 to +35 of *CC*(-61.5), a promoter that carries a consensus DNA site for CRP centered 61.5 bp upstream of the *pmelR* core promoter elements (9). Expression from *CC*(-61.5) is completely dependent on the interaction between α CTD and AR1 of CRP (3). Strain RLG4651 and plasmids pSR/*lacP1* and pRW50/*lacP1* contain positions -140 to +63 of the wild-type *lacP1* promoter (15, 16). Plasmid pSR/*lacUV5*(-140/63), which was used to generate the DNA fragment used in the experiments described in Fig. 4, contains positions -140 to +63 of the *lacUV5* promoter and includes a functional CRP-binding site (15, 16). The *lacUV5* promoter is identical to the *lacP1* promoter except for two substitutions in the -10 hexamer that improve interactions with the σ subunit of RNAP holoenzyme and make the promoter CRP independent without altering the positioning of α CTD or CRP within the initiation complex (15). Plasmids pSR/*lacUV5* and

pRW50/*lacUV5*, used in the experiments described in Fig. 2, contain positions -59 to +37 of the *lacUV5* promoter and do not contain a functional CRP-binding site.

Measurement of β -galactosidase activity. Cultures were inoculated to an A_{600} of approximately 0.007 and grown to mid-log phase (A_{600} of approximately 0.35 to 0.40) at 37°C with vigorous aeration in L broth (20 g of tryptone, 10 g of yeast extract, and 10 g of NaCl per liter) containing antibiotics where appropriate. β -Galactosidase activities were determined by the method of Miller (19). Results presented are averages of at least three independent assays and are shown with standard deviations.

Protein purification. N-terminally hexahistidine-tagged α subunit derivatives were prepared and reconstituted into RNA polymerase as described previously (23). Wild-type CRP and CRP HL159 were purified by the method of Ghosaini et al. (10) from M182 Δ crp cells transformed with plasmid pDCRP and pDCRP HL159, respectively.

In vitro transcription assays. Reactions (25- μ l final volume) were performed with 0 to 12.5 nM RNAP, 20 nM CRP, 0.2 mM cAMP, 0.2 nM supercoiled plasmid templates, 200 μ M ATP, 200 μ M CTP, 200 μ M GTP, 10 μ M UTP, and 5 μ Ci of [α -³²P]UTP in 100 mM KCl-40 mM Tris-acetate (pH 7.9)-10 mM MgCl₂-1 mM dithiothreitol-100 μ g of bovine serum albumin per ml as described previously (23). Templates were prepared using a QIAgen miniprep kit and contained promoters cloned as *EcoRI-HindIII* fragments into plasmid pSR. Reactions were started by the addition of RNAP, and after 15 min at 22°C, products were analyzed by denaturing gel electrophoresis and quantified by phosphorimaging (Molecular Dynamics) with ImageQuant software. RNAP preparations were used at 2.4 nM (wild-type RNAP), 2.2 nM (α EA261 RNAP), 12.5 nM (α RA265 RNAP), or 2.6 nM (α VA287 RNAP), concentrations that resulted in the same amounts of transcription from the *lacUV5* promoter of pSR/*lacUV5* in the absence of CRP.

DNase I footprinting. DNase I footprinting studies of purified CRP, CRP HL159, and reconstituted RNAP binding to a *PstI-HindIII* fragment of pSR/*lacUV5*(-140/63) were performed as described previously (2). The template strand was end labeled at the *HindIII* end using [γ -³²P]ATP. Products of Maxam-Gilbert G + A sequencing reactions were used as markers.

RESULTS

Residues of α CTD important for activation by CRP at *CC*(-61.5). (i) In vivo transcription assays. (a) Merodiploid

strains. As an initial screen to identify residues of α CTD important for activation of a class I CRP-dependent promoter, we analyzed the effects of 69 alanine substitutions, spanning residues 255 to 329 of α , in vivo in merodiploid assays (8, 23, 25). A reporter strain carrying a single-copy chromosomal $CC(-61.5)::lacZ$ fusion was transformed individually with plasmids encoding α derivatives, and levels of β -galactosidase expression were determined (Fig. 1A).

Overproduction of α derivatives with alanine substitutions at positions 258, 259, 261, 271, 285 to 288, 290, 294, and 317 decreased CRP-dependent transcription from the $CC(-61.5)$ promoter by 20 to 50%, with substitution of V287 and E261 causing the largest decreases (Fig. 1A). These positions can be divided into three groups (6). Residues T285, E286, V287, E288, L290, and R317 cluster on one face of α CTD and correspond to the 287 determinant previously shown to be required for class II CRP-dependent transcription (Fig. 1B) (23). Residues D258, D259, E261, and K271 cluster on the opposite face of α CTD and correspond to the 261 determinant previously shown to be required for class I CRP-dependent transcription at the *lacP1* promoter (6, 25) (see below). The remaining residue, N294, is part of the 265 determinant previously shown to be required for both class II CRP-dependent transcription (6, 23) and class I CRP-dependent transcription at the *lacP1* promoter (6, 20, 25). (Although N294 is not critical for UP element-dependent transcription at *rmB P1* [8], it contacts the DNA backbone in an α -DNA complex [29].) The results indicate that the side chains of residues 258, 259, 261, 271, 285 to 288, 290, 294, and 317 of α may make interactions that are important for class I CRP-dependent transcription at $CC(-61.5)$.

Overproduction of certain alanine-substituted α derivatives, most notably TA263 and KA297, increased CRP-dependent transcription from the $CC(-61.5)$ promoter. T263 and K297 are surface exposed in (or adjacent to) the 265 determinant important for α CTD-DNA interactions (8, 20). Preliminary results suggest that these residues are involved in α CTD- α CTD interactions that compete with α CTD-DNA interactions in the activation complex and that the alanine substitutions increase CRP-dependent transcription by eliminating this competition (H. Chen and R. H. Ebright, unpublished results). It is also possible that these residues in the wild-type protein interfere directly with α CTD-DNA interactions at $CC(-61.5)$.

(b) Haploid strains. In the merodiploid assays described in the previous section, the effects of substitutions in the plasmid-encoded α subunits were moderated by the presence of wild-type α subunits encoded by the chromosomal *rpoA* gene (8, 23, 25). To confirm the effects of the substitutions that caused the largest defects in the merodiploid assays, VA287 and EA261, we also performed in vivo assays in strains haploid for mutant *rpoA* (Fig. 2A). In these experiments, we measured transcription from a plasmid-borne $CC(-61.5)::lacZ$ fusion in strains in which VA287 or EA261 mutant *rpoA* alleles were substituted on the chromosome for the wild-type *rpoA* allele.

The results indicate that alanine substitutions at V287 and E261 reduced CRP-dependent transcription at $CC(-61.5)$ by 83 and 64%, respectively (Fig. 2A). As expected, the defects in promoter activity were greater than those observed in the presence of plasmid-borne mutant *rpoA* alleles expressed in *trans* to wild-type *rpoA*. Control experiments indicated that the VA287

and EA261 substitutions had little or no effect on CRP-independent transcription from the *lacUV5* promoter (Fig. 2A).

(ii) In vitro transcription assays. While the effects of alanine substitutions in α CTD in vivo suggested that the 261 and 287 determinants are important for CRP-dependent transcription from $CC(-61.5)$, these results did not prove that the effects were direct. Therefore, we measured the effects of the EA261 and VA287 substitutions on CRP-dependent transcription at $CC(-61.5)$ in vitro using reconstituted RNAP derivatives (Fig. 2B). We also examined the effects of the RA265 substitution, the substitution in α CTD that causes the largest defect in DNA binding to UP elements (8, 20).

The VA287 substitution resulted in an 89% reduction in CRP-dependent transcription at $CC(-61.5)$ in vitro. In contrast, neither the EA261 substitution nor the RA265 substitution caused significant changes in CRP-dependent transcription at $CC(-61.5)$ in vitro under the assay conditions used. None of the RNAP derivatives tested produced a detectable level of transcript from the $CC(-61.5)$ promoter in the absence of CRP. However, as the assays used RNAP concentrations that gave identical yields of transcript from the CRP-independent *lacUV5* promoter, the observed defects are most likely attributable to decreases in activation by CRP. These results confirm the direct role of the 287 determinant in CRP-dependent transcription from $CC(-61.5)$.

Residues of α CTD important for activation by CRP at *lacP1*.

Previous investigations of activation of promoters where CRP binds at position -61.5 focused on the *E. coli lacP1* promoter (20, 25, 31). These studies employed both random and site-directed mutagenesis approaches and concluded that key determinants of α CTD for class I CRP-dependent activation of *lacP1* are the 261 determinant (25) and the 265 determinant (20, 25). The previous studies did not identify the 287 determinant (20, 25). In order to compare directly the role of the key residues in each determinant in class I CRP-dependent transcription activation at *lacP1* and $CC(-61.5)$, we measured transcription from *lacP1* in vivo and in vitro using the same experimental conditions as for our analysis of $CC(-61.5)$.

The effects of overproducing α derivatives with single alanine substitutions were determined using a reporter strain carrying a single-copy chromosomal *lacP1::lacZ* fusion (merodiploid assays; Fig. 3A). The reporter strain was transformed individually with plasmids encoding α derivatives with single alanine substitutions at positions 287, 265, and 261, and levels of β -galactosidase expression were measured. All three substitutions caused small but reproducible decreases in class I CRP-dependent transcription at *lacP1*.

Next, transcription from a *lacP1::lacZ* fusion carried on a plasmid was measured in strains in which VA287 or EA261 mutant *rpoA* alleles were substituted on the chromosome for the wild-type *rpoA* allele (haploid assays; Fig. 3B). The results confirm that the VA287 and EA261 substitutions result in defects in class I CRP-dependent transcription at *lacP1* (reducing activity by 61 and 55%, respectively). As observed with $CC(-61.5)$, the defects in promoter activity in these strains, haploid for mutant *rpoA*, were greater than observed in the presence of plasmid-borne mutant *rpoA* alleles expressed in *trans* to wild-type *rpoA*. (Since RA265 α subunits do not support growth in the absence of wild-type α [8], it was not pos-

(A)

(B)

FIG. 1. Residues of α -CTD important for activation by CRP at *CC(-61.5)*: in vivo transcription assays of merodiploid strains. (A) RLG4650 cells carrying a chromosomal *CC(-61.5)::lacZ* fusion were transformed with derivatives of plasmids pHTf1 α (substitutions at 255 to 271 and 302) or pREII α (substitutions at remaining positions). Each plasmid encoded an α derivative with a single alanine substitution between residues 255 and 329 as indicated in the figure. β -Galactosidase activities are expressed as percentages of the activity obtained with cells transformed with plasmids encoding wild-type α (100% = 143 Miller units). Positions at which alanine substitution decreased activity by >20% are indicated by arrows. Residues 267, 272, 274, 308, 324, and 327 are alanines in the wild-type protein. The β -galactosidase activity obtained with cells transformed with a vector-only control plasmid (pDU9) is indicated by an asterisk. (B) Structure of α -CTD (12), showing side chains identified as critical for CRP-dependent transcription at *CC(-61.5)*. Two views of the structure, related by a 180° rotation on the vertical axis, are shown. Side chains of residues belonging to the 261, 287, and 294 determinants are shown in blue, red, and green, respectively.

FIG. 2. Residues of α CTD important for activation by CRP at *CC(-61.5)*: *in vivo* transcription assays of haploid strains and *in vitro* transcription assays. (A) Strains haploid for *rpoA* (WAM106, wild-type chromosomal *rpoA*; WAM140, chromosomal *rpoA*-EA261; and WAM144, chromosomal *rpoA*-VA287) were transformed with the *CC(-61.5)::lacZ* fusion plasmid pRW50/*CC(-61.5)* or the *lacUV5::lacZ* fusion plasmid pRW50/*lacUV5*. β -Galactosidase activities are expressed as percentages of the activity obtained with transformed WAM106 [100% = 340 Miller units for *CC(-61.5)* and 1156 Miller units for *lacUV5*]. (B) Multiple-round *in vitro* transcription experiments were performed using supercoiled pSR/*CC(-61.5)* template, wild-type CRP, and RNAP reconstituted with hexahistidine-tagged α derivatives containing alanine substitutions at the indicated positions. Purified RNAPs were normalized as described in Materials and Methods. Values (with standard deviation) are expressed as percentages of the yield of transcript with wild-type RNAP and wild-type CRP.

sible to replace the chromosomal *rpoA* allele with *rpoA* RA265.)

Finally, we measured CRP-dependent transcription activation from the *lacPI* promoter *in vitro* using RNAP derivatives reconstituted with α derivatives carrying the VA287, RA265, or EA261 substitution (Fig. 3C). The results indicate that all three substitutions directly cause defects in class I CRP-dependent transcription at *lacPI*, with the VA287 substitution result-

ing in the most severe defect (reducing activity by 84%). In the absence of CRP, *lacPI* promoter activity was very low, preventing precise quantitation. Nevertheless, no effects of the α mutants on basal transcription were detected, and the defects observed in the presence of CRP are likely to result from defects in activation rather than basal transcription, since the assays used RNAP concentrations that gave identical yields of transcript from the CRP-independent *lacUV5* promoter.

FIG. 3. Residues of α CTD important for activation by CRP at *lacPI*. (A) RLG4651 (chromosomal *lacPI::lacZ* fusion) was transformed with plasmids encoding mutant α derivatives as indicated. β -Galactosidase activities are expressed as percentages of the activity obtained with cells transformed with plasmids encoding wild-type α (100% = 2,860 Miller units). (B) Strains haploid for *rpoA* (WAM106, wild-type chromosomal *rpoA*; WAM140, chromosomal *rpoA*-EA261; and WAM144, chromosomal *rpoA*-VA287) were transformed with the *lacPI::lacZ* fusion plasmid pRW50/*lacPI*. β -Galactosidase activities are expressed as percentages of the activity obtained with WAM106 transformed with pRW50/*lacPI* (100% = 6,770 Miller units). (C) Multiple-round *in vitro* transcription experiments were performed using supercoiled pSR/*lacPI* template, wild-type CRP, and RNAP reconstituted with hexahistidine-tagged α derivatives containing alanine substitutions at the positions indicated. Purified RNAPs were normalized as described in Materials and Methods. Values (with standard deviation) are expressed as percentages of the yield of transcript with wild-type RNAP and wild-type CRP.

FIG. 4. Substitutions in the 287 determinant alter the DNase I footprint of a class I transcription initiation complex. DNase I footprints of complexes containing the *lacUV5* promoter (end labeled on the template strand), CRP or CRP derivative (100 nM), and reconstituted wild-type (wt) or mutant RNAPs. A, TA285:VA287 α RNAP; B, VA287:RA317 α RNAP. Lane 1 of the autoradiogram shows a Maxam-Gilbert G + A sequencing reaction. The shaded bars indicate protection by α , CRP, and the rest of RNAP. Arrows indicate DNase I-hypersensitive sites at positions -45 and -46, immediately downstream of the CRP binding site. The lower part of the figure shows a close-up of this part of the gel.

DNase I footprinting of a class I transcription initiation complex. We used DNase I footprinting to examine the effects of single and multiple substitutions within the 287 determinant of α CTD on the architecture of a class I CRP-RNAP-promoter complex (Fig. 4). The *lacUV5* promoter was used in these experiments in order to increase occupancy of the promoter by RNAP in the absence of CRP- α CTD contacts (15).

In ternary complexes containing both wild-type CRP and wild-type RNAP, protection was observed spanning the CRP and RNAP binding sites (Fig. 4, lane 4). Positions -45 and -46, which are located at the junction between the CRP and α CTD binding sites and are hypersensitive to DNase I cleavage in the complex containing only CRP (Fig. 4, lane 3, arrows), are protected in the ternary complex containing CRP and RNAP (Fig. 4, lane 4, arrows). In contrast, positions -45 and -46 are not protected in ternary complexes containing a CRP mutant with a disruption in AR1 (CRP HL159; Fig. 4, lane 7, arrows). This indicates that protection of positions -45 and -46 is diagnostic of a productive AR1- α CTD interaction. Protection from DNase I cleavage at the junction of the CRP and α binding sites is also diagnostic of productive AR1- α CTD interaction at the class II CRP-dependent promoter *galP1* (2).

Protection of positions -45 and -46 is also reduced in

FIG. 5. Model for class I and class II CRP-dependent promoter complexes. (A) At a class I CRP-dependent promoter with a CRP binding site centered at approximately -61.5 [e.g., *CC(-61.5)* or *lacP1*], the α CTD 287 determinant interacts with AR1 of the downstream subunit of the CRP dimer, the 265 determinant interacts with σ (4). The relative contribution of the α CTD 265 determinant to complex formation (hatched) varies, depending on promoter sequence. (B) At a class II CRP-dependent promoter [e.g., *CC(-41.5)* or *galP1*], the α CTD 287 determinant interacts with AR1 of the upstream subunit of the CRP dimer bound at -41.5, and the 265 determinant interacts with DNA. A second activating region of CRP (AR2) interacts with α NTD at class II CRP-dependent promoters (21).

complexes containing RNAPs with substitutions in the 287 determinant of α CTD (TA285:VA287 and VA287:RA317; lanes 5 and 6). This result is consistent with direct involvement of the 287 determinant in AR1- α CTD interactions.

DISCUSSION

Our work defines three determinants of α CTD for class I CRP-dependent transcription at *CC(-61.5)* and *lacP1*: the 287 determinant, the 265 determinant, and the 261 determinant.

The 287 determinant is required for CRP-dependent transcription in vivo and in vitro at both class I and class II CRP-dependent promoters (23; this work). Substitutions within the 287 determinant do not affect α CTD-DNA interactions (23) but do affect cooperative DNA binding by CRP and α (23) and the architecture of transcription initiation complexes at class I CRP-dependent promoters (Fig. 4). We propose that the 287 determinant of α CTD constitutes the contact surface for AR1 of CRP at both class I and class II CRP-dependent promoters (Fig. 5). We suggest that the 287 determinant can also mediate α CTD-CRP interactions at promoters where the DNA site for CRP is located further upstream than -61.5 (N. J. Savery, unpublished data). The fact that the 287 determinant was not detected in previous searches for residues of α CTD affecting CRP-dependent activation of the *lacP1* promoter (20, 25, 31) most likely reflects the fact that single substitutions in the 287 determinant have only modest effects on transcription from *lacP1* when expressed from plasmids in *trans* to wild-type α (Fig. 3A).

The 265 determinant is also required for CRP-dependent transcription at both class I and class II CRP-dependent promoters (20, 23, 25, 31; this work). This determinant mediates both sequence-specific and nonspecific α CTD-DNA interactions (8). We propose that the 265 determinant mediates in-

teractions between α CTD and the DNA segment adjacent to the binding site for CRP at both class I and class II promoters and that these interactions contribute to the overall stability of the initiation complex. However, our results indicate that the relative contributions of residues within the 265 determinant to CRP-dependent transcription vary from promoter to promoter; for example, R265 contributes significantly to class I CRP-dependent transcription activation at *lacP1* but not at *CC*(-61.5).

The promoter specificity of the contribution of individual residues within the 265 determinant could derive from differences in the α -binding potential of the DNA sequences adjacent to the CRP binding site in different promoters, from differences in the contribution of the kinetic step affected by α CTD-DNA interactions to the overall rate of transcription initiation at different promoters, and/or from subtle variations in the geometry of the specific initiation complex at different promoters. Consistent with the possibility that DNA sequence variation can alter details in DNA recognition by α CTD, alanine substitutions in the DNA-binding determinant of α CTD differentially affect transcription activation of the *rrnB* P1 versus *rrnE* P1 promoters by the transcription factor Fis (1).

The 261 determinant is required for class I CRP-dependent transcription but, with the possible exception of residue K271, not for class II CRP-dependent transcription (23). Substitutions within the 261 determinant cause defects in class I CRP-dependent transcription at *CC*(-61.5) and *lacP1* in vivo and, at *lacP1*, in abortive initiation and multi-round transcription experiments in vitro (Fig. 1, 2, and 3) (25). The 261 determinant has been proposed to interact with the σ subunit of RNAP at class I CRP-dependent promoters (where α CTD binds adjacent to the -35 hexamer) (6). In support of this proposal, substitution of E261 does not affect α CTD-DNA interactions or cooperative DNA binding by CRP and α subunits (23), and RNAP containing α EA261 is defective for UP element function at promoters with strong proximal UP element subsites (W. Ross and R. L. Gourse, unpublished data; H. Chen and R. H. Ebricht, unpublished data). The observation that KA271 causes modest defects in class II CRP-dependent transcription in vivo and in vitro at *CC*(-41.5) remains unexplained (23), as does the larger effect of substitutions in the 261 determinant in class I CRP-dependent transcription from *CC*(-61.5) and *lacP1* in vivo than in our in vitro assay (Fig. 2 and 3) (25).

Models for class I and class II CRP-dependent transcription complexes are presented in Fig. 5. The key feature of these models is that the α CTD interaction with CRP involves the same contact surfaces when CRP is located at very different positions, i.e., upstream or downstream of α CTD (6). A similar situation has been reported recently for Fis-dependent activation at the *rrn* P1 (class I) and *proP* P2 (class II) promoters, where α CTD interactions with Fis are the same in both cases (1, 18). Furthermore, we speculate that α CTD interacts with both CRP and σ in the class I activation complex and that α CTD-AR1 and α CTD- σ interactions contribute to orienting α CTD on the DNA. Priorities for current work include a detailed analysis of the proposed interaction between the 261 determinant and σ subunit and confirmation of all proposed interactions by determination of high-resolution structures.

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