JOURNAL OF BACTERIOLOGY, Apr. 1995, p. 1712–1718 Vol. 177, No. 7
0021-9193/95/$04.00+0

Transcription Activation at the *Escherichia coli uhpT* Promoter by the Catabolite Gene Activator Protein

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Received 15 September 1994/Accepted 23 January 1995

Transport and utilization of sugar phosphates in *Escherichia coli* depend on the transport protein encoded by the *uhpT* gene. Transmembrane induction of *uhpT* expression by external glucose 6-phosphate is positively regulated by the promoter-specific activator protein UhpA and the global regulator catabolite gene activator protein (CAP). Activation by UhpA requires a promoter element centered at −64 bp, relative to the start of transcription, and activation by CAP requires a DNA site centered at position −103.5. This DNA site binds the cyclic AMP-CAP complex in vitro, and its deletion from the promoter reduces transcription activity to 7 to 9% of the wild-type level. Ten *uhpT* promoter derivatives with altered spacing between the DNA site for CAP and the remainder of the promoter were constructed. Their transcription activities indicated that the action of CAP at this promoter is dependent on proper helical phasing of promoter elements, with CAP binding on the same face of the helix as RNA polymerase does. Five CAP mutants defective in transcription activation at class I and class II CAP-dependent promoters but not defective in DNA binding or DNA bending (positive control mutants) were tested for the ability to activate transcription. These CAP^{pc} mutants exhibited little or no defect in transcription activation at *uhpT*, indicating that CAP action at *uhpTp* involves a different mechanism than that which is used for its action at other classes of CAP-dependent promoters.

The *uhpT* gene of *Escherichia coli* encodes an active transport system that enables growth on a variety of sugar phosphates (reviewed in reference 19). Expression of *uhpT* is induced by extracellular glucose 6-phosphate (Glu6P) (48) and subject to catabolite repression (2). Normal regulation of *uhpT* requires the products of the *uhpA*, *uhpB*, and *uhpC* regulatory genes upstream of *uhpT* (37, 45). UhpA is a transcription activator protein with extensive amino acid sequence similarity to the phosphate-accepting transcription activators of two-component regulatory response systems (41, 46). Both UhpB and UhpC are polytopic membrane proteins (16). The polar, carboxyl half of UhpB protein has sequence similarity to the protein kinase component of two-component regulatory systems, and UhpC has extensive sequence similarity and topological similarity to UhpT (16, 46).

Catabolite repression of *uhpT* requires the catabolite gene activator protein (CAP; also called the cyclic AMP [cAMP] receptor protein) (2). CAP is a global regulator of gene expression and activates transcription at more than 50 promoters (reviewed in references 4 and 17). CAP functions in the presence of the allosteric effector, cAMP, by binding to specific DNA sites located in or near CAP-dependent promoters. CAP binding results in the bending of DNA by 90° (36), and it has been proposed that activation of transcription occurs through direct contact between CAP and RNA polymerase (29). Under non-catabolite-repressing conditions (e.g., growth with glycerol as the carbon source), CAP is predominantly in the cAMP-liganded, active conformation. The cellular level of cAMP in *E. coli* is influenced by the nature of the carbon source, in part through the inhibition of adenylate cyclase activity by glucose (34). Under catabolite-repressing conditions (growth with glucose), the level of the cAMP-CAP complex declines and the activities of CAP-dependent promoters decrease accordingly.

Comparison of the sequences of CAP-dependent promoters showed that the consensus CAP-binding site is a 22-bp inverted repeat sequence, although no naturally occurring CAP sites are fully symmetrical (7, 12). CAP-dependent promoters can be divided into three classes (8, 43, 51). Class I and class II promoters require only CAP for transcription activation. In class I CAP-dependent promoters, the site at which CAP binds to DNA is upstream of the site for RNA polymerase binding. Functional CAP-binding sites on DNA can be located at various distances from the transcription start point, centered at or near position −61.5, −72.5, −82.5, or −92.5, so long as CAP and RNA polymerase bind on the same face of the DNA helix. The degree of stimulation decreases as the distance increases (11, 42, 43). The prototype class I promoter is the *lacP1* promoter (CAP-binding site centered at position −61.5).

In class II CAP-dependent promoters, the CAP-binding site overlaps the RNA polymerase-binding site on DNA, apparently allowing CAP to replace the RNA polymerase-binding determinants at the −35 region. The prototype class II promoter is the *galP1* promoter, for which the CAP-binding site is centered at −41.5. Class III CAP-dependent promoters require a regulon-specific activator protein in addition to CAP for transcription activation. In class III promoters, the CAP site can be located at various distances from the transcription start point and typically is more than 90 bp from the transcription start point. Examples of class III promoters include the *araBAD* and *malK* promoters (18, 32).

Recent work has shown that substitutions at amino acids 156 to 162 of CAP strongly decrease transcription activation at class I and class II CAP-dependent promoters but do not markedly affect the specific binding of CAP to its DNA targets or its ability to induce a sharp bend in DNA (positive control phenotype) (3, 6, 9, 26, 30, 47, 50–52). This part of CAP has been designated the activating region of CAP, and it has been proposed that transcription activation at these CAP-dependent

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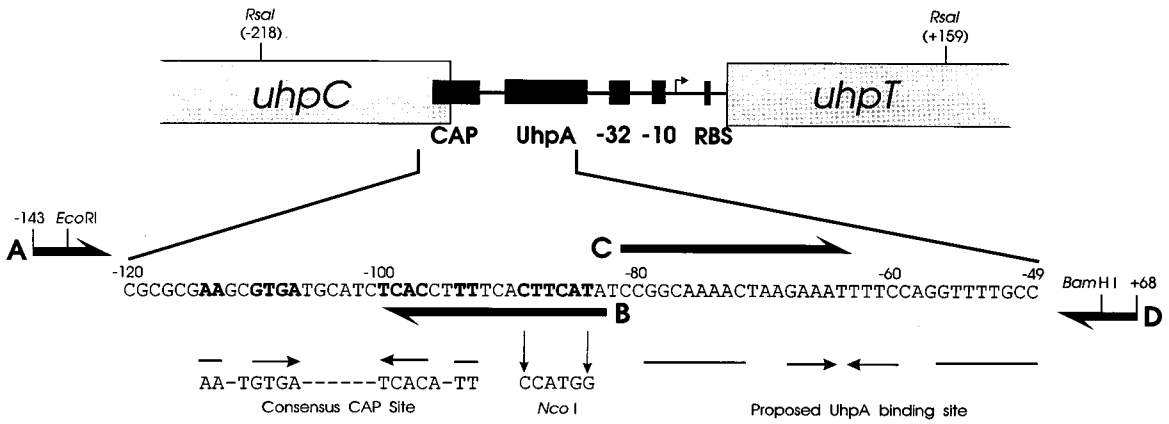

FIG. 1. Sequence of the *uhpT* promoter region. The locations of the ribosome binding site (RBS), the transcription start point (right-angled arrow), the −10 region, the short inverted repeat centered at position −32.5, the proposed site for UhpA action centered at −64, and the DNA site for CAP are indicated (21). The nucleotide sequence of the CAP region and part of the −64 element is presented and compared with the consensus sequence for a CAP-binding DNA site. The sequence changes that result from substitution of the *Nco*I linker at positions −89 to −84 are shown. PCR primers used to generate promoter fragments for gel shift assays (A, B, C, and D) are indicated.

promoters involves a direct protein-protein interaction between this part of CAP and RNA polymerase (6, 26, 47, 51, 52).

The *uhpT* promoter is a class III CAP-dependent promoter, requiring UhpA in addition to CAP for transcription activation. The sequence elements that constitute the *uhpT* promoter have been investigated by deletion and linker replacement mutagenesis (21). At least four sequence elements are required for maximal expression, a −10 region, an inverted repeat centered at position −32.5, an inverted repeat centered at position −64 (which appears to be the DNA site for UhpA), and an inverted repeat centered at position −103.5 that matches the consensus sequence for CAP-binding DNA sites (Fig. 1). In this report, we show that CAP binding to the −103.5 region strongly stimulates *uhpT* transcription and that this activation is dependent on DNA helical phasing. We also show that amino acids 156 to 162, at the activation surface of CAP, are critical for transcription activation at a class I CAP-dependent promoter but not for expression of *uhpT*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. *E. coli* K-12 strains, plasmids, and bacteriophages used in this work are listed in Table 1.

Electrophoretic mobility shift assays. Four PCR primers (A, B, C, and D) were chosen to amplify two fragments of the *uhpT* promoter; one fragment contained the CAP-binding site, and the other fragment contained the proposed UhpA-binding site and the promoter elements centered at −32 and −10 (Fig. 1). PCR primers A and D were designed to introduce unique restriction sites in the two promoter fragments for easy labeling of DNA. Both PCR fragments were labeled with ^{32}P by digestion with either *Eco*RI or *Bam*HI, agarose gel purification, and filling in of sticky ends with [α-^{32}P]dATP and [α-^{32}P]dTTP by using the Klenow fragment of DNA polymerase I (35). Fragments in unlabeled form were also used as competitor DNA in a gel shift assay. CAP was isolated from *E. coli* K-12 strain TM066 (pYZCRP) (52, 53). Purification of CAP was carried out in a single step by cAMP affinity chromatography (49). Reaction mixtures (15 μl) contained 3 ng of ^{32}P-labeled 54-bp DNA fragment that contained the CAP-binding site (ca. 10^5 cpm), 0 or 1.4 μM CAP, 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 8.0), 500 mM potassium glutamate, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, and 0.5 mM cAMP. Samples were incubated for 20 min at 25°C. Competitor DNA, when present, was added to the tube and dried by lyophilization before the addition of binding assay components. Prior to samples being loaded, all gels were preelectrophoresed until the current dropped to a constant value. Immediately before being loaded for electrophoresis, 1.5 μl of 50% glycerol with 0.25% xylene cyanol and 0.25% bromophenol blue in the same buffer was added. Electrophoresis was performed at 15 V/cm through an 8% polyacrylamide slab gel in buffer of 90 mM Tris–90 mM borate (pH 8.0), 2 mM EDTA, and 0.5 mM cAMP. Electrophoresis was carried out for 140 min at 16°C. Gels were dried and autoradiographed on Du Pont Reflection NEF-496 film.

Mutants with altered spacing between the CAP site and the promoter. Plasmid pTM705 carries the *uhpT* promoter region from positions −218 to +159 on a 377-bp *Rsa*I fragment with an *Nco*I linker substitution for the wild-type sequence from positions −89 to −84 (Fig. 1). This altered *uhpT* promoter behaves in wild-type fashion (21). Derivatives of plasmid pTM705 in which the spacing in the *uhpT* promoter was altered by −4 or +4 bp were constructed by digestion with *Nco*I and subsequent ligation after the removal of 5′ overhanging ends with mung bean nuclease or after the 5′ overhanging ends had been filled in with the Klenow fragment of DNA polymerase I and deoxynucleoside triphosphates (dNTPs), respectively (35). The promoter derivative in which the spacing was altered by +10 bp was constructed by insertion of the self-complementary linker 5′-CATGCTGCAG-3′ at the *Nco*I site of plasmid pTM705. Promoter derivatives in which the spacing was altered by +8, +16, +19, +22, +26, +29, and +32 bp were constructed by insertion of the self-complementary linker 5′-CATGGCGC CTAGGCTAGCTTAAGCTAGCCTAGGCGC-3′ at the *Nco*I site of plasmid pTM705; digestion with *Nar*I, *Avr*II, *Nhe*I, or *Afl*II; and subsequent religation directly or after the 5′ overhanging ends had been removed by mung bean nuclease or filled in with the Klenow fragment of DNA polymerase I and dNTPs. The sequence alterations in *uhpTp* to generate helically phased CAP-binding sites are shown in Fig. 2. For each promoter derivative, the nucleotide sequence of the *uhpT* promoter region was verified by using the Sequenase 2.0 reaction system (United States Biochemical Corp., Cleveland, Ohio).

TABLE 1. Bacterial strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Relevant characteristics	Reference or source
Strains		
MC4100	Δ*lacU169 araD139 deoC1 flbB5301 ptsF25 rbsR rpsL150*	38
G1044	F$^-$ *his rpsL relA* Δ*crp*::Cm Δ*cya854 ilv*::Tn*10*	S. Garges
JD010	MC4100 Δ*crp*::Cm	This work
JD020	MC4100 Δ*cya ilv*::Tn*10*	This work
JD030	MC4100 Δ*crp*::Cm *lac*$^+$	This work
SA2777	Δ*crp his relA*	S. Adhya
TM066	SA2777 λRZ5(Km)P_TRsal	This work
Plasmids		
pRS415	Ap^r; *lac* operon fusion vector	39
pTM705	pRS415 with *uhpT* promoter that contains an *Nco*I site at position −89 fused to *lacZ*	21
pYZCRP	Ap^r; *crp*	52, 53
Phages		
λRZ5	Ap^s *lacZ′* fusion vector	33
λRZ5(Km)P_TRsal	Km^r; *uhpT-lacZ*	16

wild-type	CA**CTTCAT**ATCC
pTM705	CA**CCATGG**ATCC
-4 bp	CA**CG**ATCC
+4 bp	CA**CCATGCATGG**ATCC
+8 bp	CA**CCATGGCGCCATGG**ATCC
+10 bp	CA**CCATGCTGCAGCATGG**ATCC
+16 bp	CA**CCATGGCGCCTAGGCGCCATGG**ATCC
+19 bp	CA**CCATGGCGCCTACTAGGCGCCATGG**ATCC
+22 bp	CA**CCATGGCGCCTAGGCCTAGGCGCCATGG**ATCC
+26 bp	CA**CCATGGCGCCTAGGCTAGCCTAGGCGCCATGG**ATCC
+29 bp	CA**CCATGGCGCCTAGGCTACTAGCCTAGGCGCCATGG**ATCC
+32 bp	CA**CCATGGCGCCTAGGCTAGCGCTAGCCTAGGCGCCATGG**ATCC

FIG. 2. Sequence alterations in *uhpTp* to generate helically phased CAP-binding sites. Sequences inserted between the DNA binding sites for CAP and UhpA are shown in boldface and are underlined.

Measurement of *uhpT* promoter activity. Each promoter derivative was transferred as an *Eco*RI-*Bam*HI fragment into plasmid pRS415 (39) to generate a transcriptional fusion to *lacZ*. Each *uhpT-lacZ* fusion was transferred by homologous recombination to bacteriophage λRZ5 (33, 38), and the resulting *uhpT-lacZ* fusion-bearing bacteriophage was used to lysogenize strain MC4100. Lysogens were verified to be single-copy lysogens by determination of Glu6P-induced β-galactosidase activities for at least six independent isolates. Each monolysogen was made Δ*crp* (Cm^r) or Δ*cya* (Tc^r) by transduction with G1044 P1 lysates.

Assays of β-galactosidase activity were performed by a modification of the method of Miller (21, 22). Cultures were incubated with constant agitation at 37°C in 0.2 ml of minimal medium A supplemented with 1.5 mM $MgSO_4$, 0.25% Casamino Acids, ampicillin (50 μg/ml), and 0.4% glycerol or glucose in polystyrene microtiter plates (doubling time, ca. 40 min). When cultures reached an optical density at 650 nm (OD_{650}) of 0.1, Glu6P was added to 250 μM, and cultures were incubated an additional 40 min. A 150-μl portion of each culture was transferred to a polypropylene microtiter plate and permeabilized by the addition of 10 μl of chloroform and 10 μl of 0.1% sodium dodecyl sulfate. Fifty microliters of each permeabilized culture was transferred back to a polystyrene microtiter plate that contained 100 μl of Z buffer and 2 mM *o*-nitrophenyl-β-galactopyranoside in each well. The change in OD_{415} over 3 min at 37°C was continuously measured with a Thermomax microplate kinetic reader (Molecular Devices, Menlo Park, Calif.). Units were defined as follows: 1 unit = $(OD_{415} - OD_{650}) \times OD_{650}^{-1}$.

RESULTS

Binding of CAP to the *uhpT* promoter. CAP binding to the *uhpT* promoter region was demonstrated by gel electrophoretic mobility shift assays with a ^{32}P-labeled DNA fragment that corresponded to positions −136 to −83 (Fig. 3, lane B). An identical gel shift was seen with extracts from crp^+ strain JM101 instead of CAP, but no shift was seen with extracts from Δ*crp* strain XE65.2 (data not shown). A titration experiment was performed with final CAP concentrations of 140, 430, and 720 nM and 1.4 and 2.2 μM. A single shifted band of the same mobility was observed with each concentration of CAP, indicating the presence of only one CAP-binding sequence in this DNA region (data not shown). Sequence specificity of the CAP-binding reaction was demonstrated by competition upon the addition of increasing amounts of unlabeled DNA fragment of the same sequence as the probe (positions −143 to −83) (Fig. 3, lanes C through E) and by the absence of competition upon the addition of excess unlabeled DNA fragment that contained the remainder of the *uhpT* promoter (positions −84 to +159) (lanes F through H). When the *uhpTp* fragment that contained the sequence from −81 to +68 was ^{32}P labeled and purified CAP was added to the binding reaction, no mobility shift was observed (data not shown).

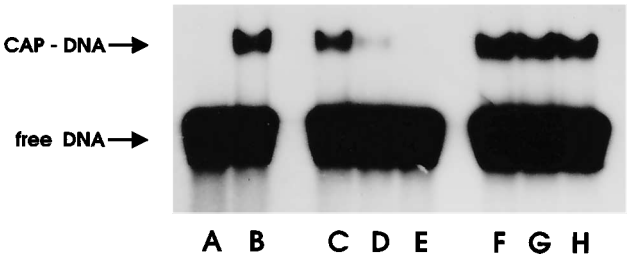

FIG. 3. Binding of CAP to DNA that carries the *uhpT* promoter. The ^{32}P-labeled PCR product corresponds to positions −136 to −83. Lane A, 0 nM CAP; lane B, 1.4 μM CAP; lanes C to E, 1.4 μM CAP plus 25-, 125-, and 250-fold excess, respectively, of unlabeled DNA fragment that corresponds to promoter positions −143 to −83; lanes F to H, 1.4 μM CAP plus 25-, 125-, and 250-fold excess, respectively, of unlabeled DNA fragment that corresponds to positions −81 to +68 of *uhpT*.

Effects of deletions of the CAP-binding region in the *uhpT* promoter. To examine the effects of deletions of the CAP-binding site in the *uhpT* promoter, promoter derivatives with upstream deletions that removed part or all of the *uhpT* DNA site for CAP (endpoints at positions −105 or −79) (21) were assayed in Glu6P-induced cells grown with Casamino Acids and either glycerol or glucose as carbon sources. The promoter derivative that lacked *uhp* DNA sequences upstream of −115 but retained the entire CAP-binding consensus sequence exhibited wild-type levels of transcription activity and the typical 50% decrease in activity in response to glucose-elicited catabolite repression (Fig. 4). The two promoter derivatives whose deletions extended into the CAP-binding element exhibited 11-fold-lower β-galactosidase activities than that of the wild-type promoter and complete loss of glucose-elicited catabolite repression. To ensure that this observed activation by CAP was not due to other consequences of deleting the sequence around −103.5 in *uhpTp*, the activities of these promoter deletions in isogenic crp^+ and *crp* strains were compared (Fig. 4). Without CAP-cAMP complexes in cells, the presence or absence of *uhpTp* sequence upstream of −79 did not influence promoter activity. These results indicate that both the maximal activity of the *uhpT* promoter and its response to glucose-elicited catabolite repression require the *uhpTp* DNA site for CAP.

Effects of altered spacing between the CAP site and the promoter. To examine the effects of altered spacing between the CAP-binding sequence and the remainder of the *uhpT* promoter, promoter derivatives with spacing decreased by 4 bp or increased by 4, 8, 16, 19, 22, 26, 29, or 32 bp were constructed (Fig. 2). Each deletion or insertion was made at position −86 by using an *Nco*I linker substituted for the authentic sequence from positions −89 to −84. Seven of the promoter derivatives had spacing that had been altered by nonintegral numbers of turns of the DNA helix (i.e., −4, +4, +8, +16, +19, +26, and +29 bp), and three of the promoter derivatives had spacing that had been altered by approximately integral numbers of turns of the DNA helix (i.e., +10, +22, and +32 bp). One turn of B-form DNA helix corresponds to approximately 10.5 bp (31, 44). The promoter activity of each derivative was assayed in Glu6P-induced cells grown with Casamino Acids and either glycerol or glucose as carbon sources. In all 10 promoter variants, *uhpT-lacZ* expression was completely dependent on the presence of Glu6P as an inducer (1 ± 2 Miller units of activity without Glu6P).

The seven promoter derivatives with spacing altered by non-

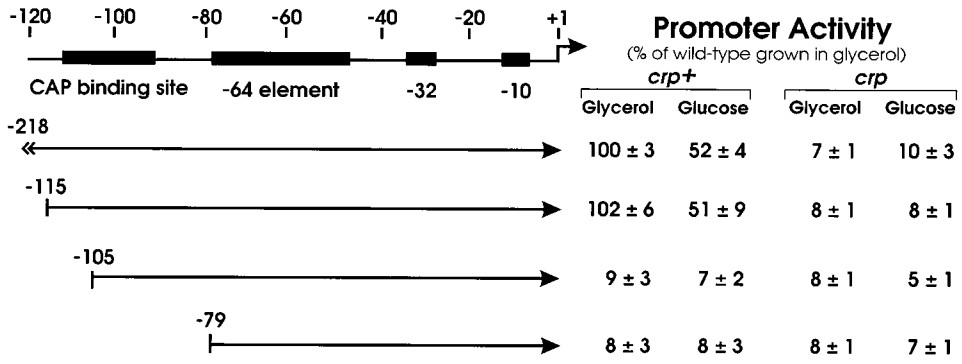

FIG. 4. Effects of deletions of the *uhpT* DNA site for CAP. The promoter sequences that remain in the promoter derivatives with upstream deletions that remove part or all of the *uhpT* DNA site for CAP are indicated (21). For each promoter derivative, promoter activities in Glu6P-induced cells grown with Casamino Acids and either glycerol or glucose as carbon sources are reported relative to the activity of the wild-type promoter in Glu6P-induced cells grown with glycerol and Casamino Acids as carbon sources (909 ± 23 units). Data are means ± standard deviations of at least six independent assays.

integral numbers of turns of the DNA helix exhibited 2 to 4% of wild-type promoter activity. The activities of several of these promoter variants were decreased in the presence of glucose (Fig. 5A). The phenotypes of these promoter derivatives were indistinguishable from that of the promoter derivative with a deletion of the DNA site for CAP.

The three promoter derivatives with spacing altered by approximately integral numbers of turns of the DNA helix (+10, +22, and +32 bp) exhibited normal glucose-elicited catabolite repression and reduced but appreciable promoter activities, decreasing from 18 to 7% of the wild-type level with increasing distance from the transcription start site. To ensure that the effects of altered spacing in *uhpTp* were due to CAP, isogenic Δ*crp* and Δ*cya* strains that contained each *uhpT* promoter derivative were constructed and assayed for Glu6P-induced β-galactosidase activity. The results shown in Fig. 5B and C indicate that active CAP-cAMP complexes were required both for stimulation of *uhpT-lacZ* transcription by the helically phased CAP binding sequence and for catabolite repression elicited by growth with glucose.

Effects of mutants of CAP defective in transcription activation at class I and class II CAP-dependent promoters. The effects on *uhpT* transcription of mutational forms of CAP defective in transcription activation at class I and class II CAP-dependent promoters but not defective in DNA binding or bending were examined (52). Plasmids that encoded wild-type CAP or the CAP^{pc} variants CAP[A156D], CAP[T158A], CAP [T158I], CAP[H159R], and CAP[G162S] were introduced by transformation into Δ*crp uhpT-lacZ* strain TM066. For each transformant, promoter activity was assayed in Glu6P-induced cells grown with glycerol and Casamino Acids as carbon sources. The transformant with wild-type CAP and the five transformants with mutants of CAP exhibited promoter activities that were equal within a factor of approximately 1.5 (Table 2). When the host strain did not carry a CAP-encoding plasmid, *uhpT* promoter activity was less than 10% of that for the strain that carried the wild-type CAP plasmid. The CAP variants were tested in vivo for *lacZ* promoter activity to compare with their activities at *uhpTp*. *lacZ* promoter activity was sharply reduced with these CAP^{pc} derivatives (Table 2).

DISCUSSION

These results demonstrate that CAP binding to the consensus CAP-binding region centered at position −103.5 is necessary for maximal activity of the *uhpT* promoter and for its response to glucose-elicited catabolite repression. The degree of transcription activation conferred by CAP was in the range of 10- to 15-fold, indicating that CAP strongly stimulates, but is not absolutely required for, transcription from this promoter. The specific transcription activator UhpA is absolutely required for transcription activity, and in its absence, no promoter activity is detected (21).

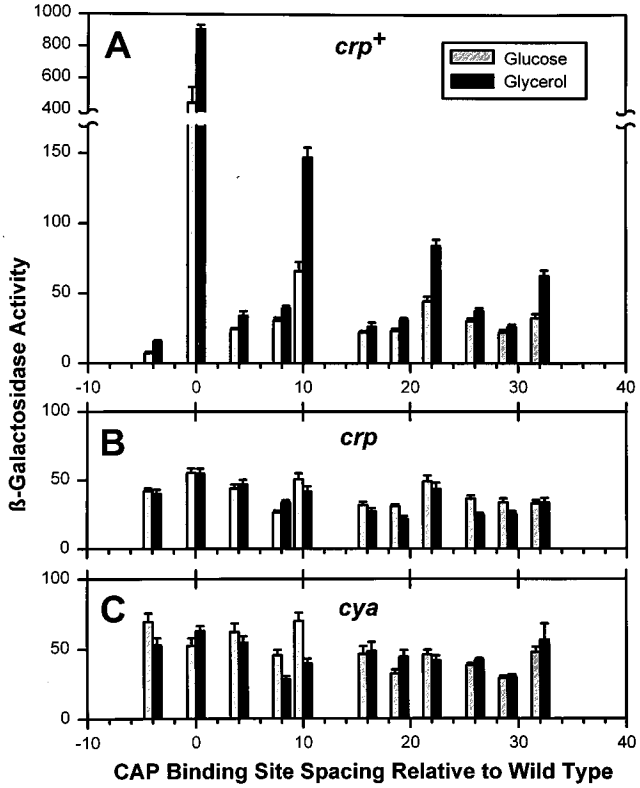

FIG. 5. Effects of altered spacing between the DNA site for CAP and the remainder of the *uhpT* promoter. Bars represent β-galactosidase expression (measured in Miller units) (21) for Glu6P-induced cells grown with Casamino Acids and either glycerol or glucose as carbon sources. In all cases, the uninduced level of activity was negligible (1 ± 2 Miller units). Activities of the wild-type *uhpT* promoter and promoters with altered CAP-binding site spacings were measured in wild-type (A), Δ*crp* (B), and Δ*cya* (C) backgrounds.

TABLE 2. Activation of *uhpT-lacZ* transcription by CAP mutants defective in activation at class I and class II CAP-dependent promoters

CAP variant	*uhpT* promoter activity[a]	*lacZ* promoter activity[b]
Vector	0.08 ± 0.02	0.03 ± 0.0
Wild-type CAP	1.0	1.0
CAP[A156D]	1.04 ± 0.13	0.04 ± 0.0
CAP[T158A]	1.24 ± 0.13	0.19 ± 0.02
CAP[T158I]	1.04 ± 0.13	0.38 ± 0.06
CAP[H159R]	1.09 ± 0.17	0.41 ± 0.05
CAP[G162S]	0.83 ± 0.08	0.11 ± 0.02

[a] The Δ*crp uhpT-lacZ* strain TM066 was transformed with plasmid pYZCRP which encoded wild-type CAP or the indicated missense mutation or with the vector plasmid. Promoter activity in each transformant determined by a β-galactosidase assay on 5 mM Glu6P-induced cells grown with glycerol and Casamino Acids as carbon sources. Promoter activity is reported relative to the activity in the presence of wild-type CAP (535 ± 60 units). Data are means ± standard deviations of at least six independent assays.

[b] The *lac* operon in strain JD030 that contained the vector plasmid or the indicated CAP variant-encoding plasmid was induced with 5 mM IPTG (isopropyl-β-D-thiogalactopyranoside). Reported promoter activities are relative to the activity seen with wild-type CAP (280 ± 20 units).

Our results show that transcription activation at *uhpTp* by CAP exhibits helical-phase dependence. CAP was not able to activate transcription at a promoter derivative in which the spacing between the CAP-binding site and the remainder of the *uhpT* promoter was increased or decreased by a nonintegral number of turns of the DNA helix. In contrast, CAP was able to activate transcription at a promoter derivative in which the spacing was increased by approximately one, two, or three helical turns. The degree of transcription activation decreased progressively as the spacing between the CAP-binding site and the remainder of the promoter increased. Expression of β-galactosidase from the *uhpT-lacZ* fusion genes driven by altered promoters was dependent on the addition of Glu6P as an inducer. This retention of regulation indicates that these promoters are still UhpA dependent and are not the result of a new, fortuitously created promoter. We conclude that transcription activation at *uhpTp* by CAP requires precise rotational positioning, not precise linear positioning, of the CAP site relative to the remainder of the promoter. Since position −103.5 should lie on the same face of the helix as positions −41, −61, −72, −82, and −92.5, positions at which CAP can activate CAP-dependent promoters, we suggest that CAP must bind to the same face of the DNA helix as RNA polymerase does in order to activate at the *uhpT* promoter. This pattern has been observed previously for promoters at which transcription activation is mediated by direct protein-protein interaction (11, 23, 24, 28, 42, 43). Although this behavior is considered diagnostic of direct protein-protein interaction (1), it is also observed for promoters at which transcription activation is mediated by DNA bending (10).

The promoter activities of variants in which the CAP-binding sequence had been rotated relative to the rest of the promoter were lower in the presence of the CAP-cAMP complex than they were in its absence. This apparent inhibition by the CAP-cAMP complex may indicate interference with proper assembly of the transcription complex by an improperly faced DNA bend.

Our results further demonstrate that positive control mutants of CAP that affected residues 156 to 162 but did not alter DNA binding or bending had no or only very slight defects in transcription activation at *uhpTp*. In contrast, the mutants tested here showed large (up to 25-fold) decreases in transcription activation at class I and class II CAP-dependent promoters (8, 26, 51, 52). It is possible that amino acids 156 to 162 of CAP interact with RNA polymerase. Some mutations in *rpoA* that delete the carboxyl-terminal region of the α subunit of RNA polymerase result in defective transcription from the CAP-dependent *lac* promoter but effective transcription from several CAP-independent promoters (14, 15). Furthermore, Heyduk et al. (13) have shown that CAP interacts with RNA polymerase in solution in the absence of promoter DNA, but not if CAP contains a missense mutation in amino acid 158. More convincing evidence is the use of protein-protein photocross-linking to show the close proximity of this activating region of CAP to RNA polymerase at the *lac* promoter (6). We conclude that the mechanism of transcription activation at *uhpTp* differs from those at other CAP-dependent promoters in its requirement for the activating region represented by amino acids 156 to 162 of CAP (3, 26, 51). Recently, it has been established that transcription activation at class II CAP-dependent promoters requires a second activation region, AR2, centered on amino acids 19 to 21 (25). Experiments with CAP derivatives with substitutions in AR2 indicate that AR2 is not essential for transcription activation at *uhpTp* (data not shown).

There are several potential models of CAP action at *uhpTp*. As with other classes of CAP-dependent promoters, CAP may make direct protein-protein contact with RNA polymerase at the *uhpT* promoter. Despite its distance from the start of transcription, promoter DNA bending might allow CAP to make contact with RNA polymerase at *uhpTp* in a nucleosome structure. Although the activation region of CAP that contains amino acids 156 to 162 does not appear to be necessary for activation at *uhpTp*, there is precedent for alternative regions of CAP making protein-protein contact with RNA polymerase. Williams et al. (47) have identified a CAP surface-exposed loop around residue 52 which may make contact with RNA polymerase at promoters with a CAP-binding site located 41.5 bp upstream of the start of transcription.

In a second model, CAP may act to stabilize UhpA bound to *uhpTp* by using a contact region other than that between amino acids 156 and 162. A precedent is the CytR repressor, which has poor affinity for its operator but whose binding is enhanced 100- to 1,000-fold when the CAP-cAMP complex is present (27). Substitutions of residues 12 to 18 or 108 to 110 of CAP lead to loss of CytR binding at the *deoP2* promoter, while CAP binding is unaltered (40). Thirdly, CAP may reposition UhpA bound to *uhpTp* rather than stabilize binding of the activator, as is seen with CAP and MalT interaction at the *malK* promoter (32). Preliminary analyses by gel retardation and DNase I footprinting techniques with both purified CAP and UhpA indicate neither cooperative binding nor repositioning of the activator.

In a fourth model, transcription activation at *uhpTp* by CAP involves a CAP-induced DNA bend. Preliminary experiments with circularly permuted DNA fragments that contain *uhpTp* suggest that CAP induces a bend at this promoter. No specific DNA sequences upstream of the CAP-binding site in *uhpTp* are necessary for transcription activation or repression. This suggests that no putative DNA bend is needed to bring a specific upstream sequence into proximity with the transcription initiation complex, as occurs at the *galP1* promoter (5), or to disrupt a repressive DNA loop, as at the *araBAD* promoter (18). An alternative role for a DNA bend by CAP at *uhpTp* is that stored bend energy may help counteract strong protein-protein or protein-DNA interactions and assist in the escape of RNA polymerase from the promoter (54). CAP may act at *uhpTp* in a similar fashion to its mechanism at the *malT* pro-

moter, where the effects of CAP on K_B and k_f are minimal but the initial steps of elongation and escape from the open complex are enhanced (20).

The *uhpT* promoter is the simplest characterized class III CAP-dependent promoter, with only one DNA site for CAP and only one region that binds the regulon-specific transcription activator UhpA, although the number and orientation of UhpA-binding sites have not been determined. As such, *uhpT* is an attractive model system for analysis of the mechanism of transcription activation at class III CAP-dependent promoters. We are currently attempting to isolate mutants of CAP, UhpA, and RNA polymerase that are specifically affected in transcription activation at *uhpTp.*

ACKNOWLEDGMENTS

We thank Yuhong Zhou, Gail Touchie, and Clifton Franklund for technical assistance and Sankar Adhya and Susan Garges for strains.

This work was supported by National Institutes of Health research grants GM38681 (R.J.K.) and GM41376 (R.H.E.). T.J.M. and J.L.D. received support from National Institutes of Health training grants CA09091 and GM08136, respectively.

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