Arginine Substituted for Leucine at Position 195 Produces a Cyclic AMP-independent Form of the Escherichia coli Cyclic AMP Receptor Protein*

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Mutant forms (CRP*) of the Escherichia coli cAMP receptor protein (CRP) that activate CRP-dependent promoters in the absence of the normal allosteric effector (cAMP) have been described. A previous report (Harman, J. G., McKenney, K., and Peterkofsky, A. (1986) J. Biol. Chem. 261, 16332–16339) detailed the properties of three CRP* mutant proteins. One protein, 220 CRP, has amino acid substitutions at positions 127 and 170 and low CRP* activity in vivo. A second protein, 222 CRP, has the amino acid substitutions present in 220 CRP and a third substitution (arginine for leucine) at position 195. 222 CRP has high CRP* activity in vivo and high apparent affinity for lacP DNA relative to the 220 CRP in vitro. In this report, we evaluate the effect of a single amino acid substitution at position 195 (leucine to arginine) on CRP activity both in vivo and in vitro. Cells (cyaacrpa perR195) containing R195 CRP were found to exhibit a CRP* phenotype, expressing a variety of CRP-dependent genes in the absence of cAMP. R195 CRP exhibited both CRP* activity in vitro and increased apparent affinity for cAMP relative to wild-type CRP. CRP titration experiments performed using an in vitro lac transcription system suggest that the isolated substitution of arginine at position 195 does not confer on CRP the high lacP affinity that distinguishes the 220 and 222 forms of CRP. These findings lead us to the conclusion that the effects of multiple mutations in CRP can be both cumulative and interactive.

The cyclic AMP receptor protein (CRP) modulates the rate of mRNA synthesis at a specific class (CRP-dependent) of promoter (reviewed in Ref. 1). In wild-type Escherichia coli, the activity of CRP-dependent promoters is controlled by the cellular concentration of cAMP which establishes the concentration of the active CRP·cAMP complex form of CRP (2). When bound to the appropriate site on DNA, CRP·cAMP enhances RNA polymerase-promoter recognition. At least three mechanisms exist to increase CRP-dependent promoter activity in cells unable to synthesize cAMP de novo (i.e. cya−).

The first involves mutation of a specific promoter to a CRP-independent class (3); the mutation affects the synthesis of mRNA from only the mutant promoter while all other members of the CRP-dependent promoter class remain dependent upon the CRP·cAMP complex for activity. The second mechanism involves mutation of RNA polymerase such that the enzyme exhibits increased recognition of some CRP-dependent promoters (4–6). The third mechanism results from mutation of the crp gene where many, if not all, CRP-dependent promoters are relieved of the normal cAMP requirement yet remain dependent upon mutant CRP.

Mutant strains of E. coli containing forms of CRP that function independently of cAMP (CRP*) have been isolated (7–19). We have recently described the genetic and biochemical characteristics of three forms of CRP* establishing: 1) the sites of mutations in crp; 2) the effects of these mutations on CRP* structure and effector specificity; and 3) the requirements of these forms of CRP to activate wild-type lacP in vitro (19). One such mutant protein, 220 CRP, has amino acid substitutions at positions 127 and 170 (19). A second mutant protein, 222 CRP, has the amino acid substitutions present in 220 CRP and an additional substitution of arginine for leucine at position 195. The results of protease digestion studies indicate that the conformations of these two forms of CRP* are different (19). In addition, 222 CRP activated a greater number of CRP-dependent promoters than did 220 CRP and exhibited increased apparent affinity for lacP DNA in the absence of cAMP. To establish the effect of the substitution at position 195 on the structure and function of CRP, independent of the effects of mutations at positions 127 and 170, we have constructed a mutant crp allele that substitutes arginine for leucine at position 195 and characterized the R195 CRP in vivo and in vitro. This study shows that R195 CRP is itself a CRP* form of CRP. The protein exhibits altered CRP conformation, altered CRP effector specificity and increased affinity for cAMP relative to the wild-type protein.

EXPERIMENTAL PROCEDURES

Bacteria and Plasmids

E. coli strain CA8445 (ΔcyaΔ45 Δcrp45) (20) transformed with pRKK24cI" (21) was the host for recombinant crp plasmids. Strain HB2154 (K12, araΔ(lac-pro)Δhi/F' proA+B+ lacF lacZ ΔM16) (22) was the recipient strain used in the mutagenesis of M13K1crp-1 phage. pRKK24cIk", which encodes a temperature-sensitive λI repressor and a TET" determinant, was a gift from D. Helinski, University of California at San Diego, La Jolla, CA. Strain C600 (r−m+, λcI+)

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1 The abbreviations used are: CRP, cAMP receptor protein; IPTG, isopropyl-1-thio-β-D-galactopyranoside.
a gift from T. Melton, North Carolina State University, Raleigh, NC. pKC30 (24), which contains the P₁ promoter and an AmpR determinant, was a gift from Y. Ho of Smith, Kline and Beckman Laboratories, Philadelphia, PA. M13K19 and HB2154 (22) were gifts of G. Winter, of the Medical Research Council, Cambridge, England.

Materials

pKl201, pHAT, pH4, pH6, and pH8 were isolated by the procedure of Norgard (25). The final step in purification was buoyant density centrifugation through CsCl gradients for plasmids pKl201 and pHAT and chromatography on Sepharose 4B following Rnase treatment and phenol extraction for plasmids pH4, pH6, and pH8. Restriction enzymes, T4 DNA polymerase, T4 DNA ligase, and the large fragment of DNA polymerase I (Klenow) were obtained from New England Biolabs, International Biotechnologies, and Bethesda Research Laboratories. NACS Prepacs columns were supplied by Bethesda Research Laboratories. These materials were used according to the protocols supplied by the manufacturers. Rnase polymerase was supplied by New England Biolabs. Plasmatal Rnase inhibitor (RNasin) was supplied by Promega Biotec. Nucleotide triphosphates were purchased from Sigma (ATP) and Pharmacia LKB Biotechnologies, Inc. (GTP, CTP, and UTP). [α-32P]UTP (800 Ci/mmol) was purchased from Du Pont-New England Nuclear. Dideoxynucleotides were purchased from P/L Biochemicals. Synthetic oligonucleotides were provided by Dr. Gerald Zorn of the Food and Drug Administration. All other chemicals were reagent grade.

Phage/Plasmid Shuttle System

To facilitate the in vitro mutagenesis of the wild-type crp allele and the subsequent expression of the mutant crp allele, the following phage/plasmid shuttle system was developed:

Step 1. -The cohesive ends of a HindIll/EcoRI M13mp18 DNA digest were made blunt with the addition of the appropriate deoxy- nucleoside triphosphates and the Klenow fragment of DNA poly-erase I. This DNA was cloned into the HpaI site of pKC30. Recombin-ant plasmids containing the polylinker of M13mp18 were identi- fied first by restriction nucleic digestion of plasmid miniprepara- tion and subsequently confirmed by DNA sequence analysis. One such plasmid, pH4, contains a tandem insert of the polylinker with the orientation:

SphI.PstI.SalI.XbaI.BamHI.SmaI.KpnI.SacI,

SacI.KpnI.SmaI.BamHI.XbaI.SalI.PstI.SphI.

Step 2. -The 942-base pair Alul fragment of pHAT containing the crp structural gene was isolated from an agarose gel, purified over a NACS Prepac column, and cloned into SmaI-linearized pH4. pHH4crp-1, contains crp in the sense orientation with respect to the P4 promoter and lacks the SmaI.PstI.Alul.EcoRI.SacI.KpnI.SacI restriction sites present in pH4.

Step 3. -The XbaI.BamHI -crp BamHI.XbaI fragment of pH4crp-1 was isolated from an agarose gel, purified over a NACS Prepac column, and cloned into the XbaI site of M13K19. M13K19crp-1 served as template DNA in the oligonucleotide-directed mutagenesis of crp as described below.

Step 4. -The double stranded replicative forms of M13K19crpR195 and M13K19crpP195 were digested with XbaI and cloned into the XbaI site of pH4. The ligation mixture was used to transform (27) strain C600 (AcI'). AMP' cells were isolated, plasmid miniprepara-tions (26) and subsequently confirmed by DNA sequence analysis.

One difference between crp-containing recombinant plasmids derived from pKC30 compared to those derived from pH4 or pH6 is the phasing of ribosomes across the vector/crp junction that initiate translation at the N gene AUG. To assess the effect of translational phasing on CRP expression from pH6crp, XbaI-linearized pH6 DNA was treated with mung bean nuclease and blunt end-ligated in dilute solution to yield pH6. pH6 has a -1 translation phase shift at the modified XbaI restriction site compared to pH4; this restores the N gene mRNA translation phasing to the crp structural gene in pH6crp mRNA to that of the original pKC50 crp constructs. The BamHI fragments of pH4crpR195 or pH4crp-1 were cloned into BamHI-linearized pH8 to yield pH4crpR195 or pH4crp-1; the ligation mixtures were used to transform strain CA8445/pRK248. The total cellular protein in extracts of CA8445/pRK248/ pH4crpR195 grown at 42 °C was approximately 3% CRP by weight.

Mutagenesis of crp

Single stranded M13K19crp-1 was isolated (28) and served as template for oligonucleotide-directed mutagenesis as described by Carter et al. (22). Second strand synthesis was primed with both the SEL2 selection primer (22) and with a 25-base oligonucleotide complementary to crp and having 2-base degeneracy at position 15.

Codon 195 (CCG) encodes proline. Codon 195 (CGG) encodes argi-nine. The oligonucleotide sequence was designed using the Staden Seqfit program (29). DNA Sequencing

Mutant M13 were identified by DNA sequence analysis using the dideoxynucleotide chain termination method (30) in reactions con-taining

| Carbon source | pH6 | pH6crpR195 | pH6crp-1 | pH6crpR195 |
|---------------|-----|------------|----------|------------|
| Lactose       | -   | +          | +        | ++(G1)     |
| +cAMP         |     |            |          |            |
| Arabinose     | +   | ++         | ++       | ++(G1)     |
| +cAMP         |     |            |          |            |
| Sorbitol      | -   |            | -        |            |
| +cAMP         |     |            |          |            |
| Galactose     | +   | ++         | +        | +          |
| +cAMP         |     |            |          |            |
| Mannitol      | +   |            | ++       | +          |
| +cAMP         |     |            |          |            |
| Ribose        | -   |            | -        |            |
| +cAMP         |     |            |          |            |
| Xylose        | -   |            | -        |            |
| +cAMP         |     |            |          |            |
| Glucose       | +   |            | ++       | ++(G1)     |
| +cAMP         |     |            |          |            |
| Maltose       | -   |            | -        |            |
| +cAMP         |     |            |          |            |
taining oligonucleotide crp.006 (19) as primer. Both crpR195 and crpP195 allelic forms of crp were identified. The entire crp was sequenced using primers LMB2 (31), crp.5, and crp.4 (19) and shown to be wild-type except for position 195.

CRP Purification

CRP was purified from extracts of strain CA8445/pRK248/pJH8crpR195 grown first at 30 °C on LB medium that contained 50 μg/ml ampicillin and 10 μg/ml tetracycline. At a culture density of A660 = 0.6, the culture incubation temperature was increased to 42 °C and maintained at this temperature for 16 h. The purification protocol which includes chromatography on phosphocellulose, hydroxylapatite, and CM-Sephadex, has been described (19). The protein was judged 95% pure by electrophoresis on 12.5% polyacrylamide-sodium dodecyl sulfate gels that had been stained with Coomassie Blue.

CRP and Protein Assay

CRP was assayed by the ammonium sulfate precipitation assay of Anderson et al. (32) as modified by Puskas et al. (14). Protein was assayed by the method of Bradford (33).

β-Galactosidase induction in strain CA8445/pRK248 containing vector, wild-type, or mutant crp alleles

Overnight cultures were grown at 30 °C in LB medium that contained ampicillin at 50 μg/ml and tetracycline at 10 μg/ml. Inocula were diluted into 37 °C medium at A600 = 0.05, grown to A600 = 0.5 and induced for β-galactosidase by dilution into prewarmed medium that was 5 mM in IPTG. Where indicated, cAMP or cGMP were present at 1 mM. After 1 h, samples were diluted 1:2 into ice-cold medium that was 5 mM in IPTG. Where indicated, cAMP or cGMP were added.

Further characterization of the R195 CRP phenotype was conducted in liquid medium. IPTG failed to induce β-galactosidase synthesis in cultures of CA8445/pRK248/pJH8 in the absence or presence of added cAMP (Table II). In cells that contained the R195 mutant plasmid pJH8crpR195, IPTG induced β-galactosidase synthesis in the presence of cAMP; cGMP did not substitute for cAMP.

Results

Table II

| Plasmid   | CRP   | β-Galactosidase activity |
|-----------|-------|-------------------------|
| pJH8      | None  | 0.032                   |
| +cAMP     |       | 0.038                   |
| +cGMP     |       | 0.031                   |
| pJH8crp-1 |        | 0.034                   |
| +cAMP     |       | 1.000                   |
| +cGMP     |       | 0.040                   |
| pJH8crpR195 | R195 | 0.046                   |
| +cAMP     |       | 1.540                   |
| +cGMP     |       | 0.561                   |

Fig. 1. Protease probe of the R195 CRP conformation. Lanes A and T contained molecular mass standards (daltons): carbonic anhydrase (30,000), trypsin inhibitor (20,100), and lactalbumin (14,400). Bracketed lanes contained either wild-type (WT) CRP or R195 CRP as indicated. Lanes B and K were loaded with samples (3 μg) of untreated CRP. Lanes C–J and L–S contained CRP that was treated with the indicated protease (circle) in the absence of cAMP (open circle) or in the presence of cAMP at 100 μM (filled circle). 12.5% polyacrylamide-sodium dodecyl sulfate gels were run according to Laemmli (43). Only the relevant portion of the gel is shown.
In cells that contained the crpR195 recombinant plasmid pJH8crpR195, IPTG in the absence of cAMP induced only slightly higher levels of β-galactosidase than cells that contained wild-type CRP; however, the addition of either cAMP or cGMP stimulated β-galactosidase synthesis in cells containing the R195 CRP. These results indicate that R195 CRP has very low CRP* activity with respect to lac-P activation in vivo as well as the altered cyclic nucleotide specificity frequently associated with CRP* forms of CRP (Table II).

Characterization of the R195 CRP in Vitro—Wild-type CRP is relatively resistant to protease digestion in the absence of cAMP and is rapidly degraded by proteases in the presence of cAMP (Refs. 34 and 35 and Fig. 1). This characteristic has been interpreted as evidence of a CAMP-induced change in CRP conformation and correlates with the CAMP-dependent CRP activity in transcriptional control (19, 34, 35). Previous studies have demonstrated that CRP* forms of CRP are more sensitive to proteases in the absence of cAMP than is wild-type CRP (15, 19). R195 CRP was degraded, in the absence of cAMP, by subtilisin, trypsin, chymotrypsin, or the Staphylococcus aureus V8 protease, a result consistent with its classification as a CRP* form of CRP (Fig. 1). Control reactions demonstrated the CAMP-dependent protease sensitivity of wild-type CRP (Fig. 1). These data, together with that of Table II, show that substitution of arginine for leucine at position 195 results in the synthesis of a form of CRP that shares, in the absence of cAMP, properties similar to the active (CRP·cAMP complex) form of wild-type CRP.

lac mRNA synthesis originating from a wild-type lac promoter (lacP) requires the CRP·cAMP complex form of wild-type CRP (19, 36, 37). We have demonstrated that three mutant forms of CRP promote lac mRNA synthesis in vitro in the absence of cAMP (19). pKL201, the template used in this transcription system, contains a CRP-independent promoter (repP) that initiates the synthesis of a 106/107-base transcript that is resolved in polyacrylamide gels from the 135/137-base transcript originating from lacP (19). rep mRNA levels serve as an internal control by which to measure either wild-type or mutant CRP effects on lacP activity. The results presented in Fig. 2A demonstrate that, unlike wild-type CRP, the R195 CRP activates lacP independently of CAMP in vitro in a CRP concentration-dependent manner. These data provide direct evidence for the CAMP-independent function of R195 CRP.

The effect of the R195 mutation on the apparent affinity of CRP for cyclic nucleotides has been determined by titrating either cAMP or cGMP in reaction mixtures that contained

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**Table III**

| CRP   | Amino acid substitution(s)* | Phenoype† | Protease sensitivity* | Apparent Kₐ,‡ | Source |
|-------|-----------------------------|-----------|-----------------------|---------------|--------|
| WT    | None                        | CRP*      | No                    | 1 µM          | Ref. 19|
| 91    | T144                        | CRP*(intermediate) | Yes       | 30 nM        | Ref. 19|
| R195  | R195                        | CRP*(weak) | Yes                   | 10 µM         | Present study|
| 220   | I127                        | CRP*(weak) | Yes                   | 2 µM          | Ref. 19|
| 222   | K170                        | CRP*(strong) | Yes                | 1 µM          | Ref. 19|
| R195  | K170                        | CRP*(strong) | Yes                | 2 µM          | Ref. 19|

*The substituted amino acid is indicated using the standard one-letter abbreviation. The numbering begins with valine as the amino-terminal amino acid.

†CRP* is used to indicate an absolute requirement for cAMP; CRP indicates CAMP independence. The phenotype indicated was determined on lactose MacConkey indicator medium using strain CA8445/pRK248.

‡Proteases include subtilisin, trypsin, chymotrypsin, and the S. auresus V8 protease.

§Apparent association constants were determined in lac transcription reactions and are assumed to reflect the affinity of CRP for either cAMP, cGMP, or DNA. Reaction conditions were varied to suit the experiment: cyclic nucleotide titrations were run under conditions where CRP* activity was minimal (50 nM CRP); CRP titrations were run in the absence of cyclic nucleotides.

‡At equilibrium, cAMP and cGMP bind wild-type CRP with similar affinity (42). The CRP·cGMP complex does not, however, activate lacP.
transcription in the absence or presence of cyclic nucleotides yet differ in their ability to promote cAMP-independent β-galactosidase synthesis in vivo. Cells containing the R195 CRP have a weak CRP* phenotype (Tables I and II) while cells containing the 91 CRP exhibit an intermediate CRP* phenotype (19). Semi-quantitative evidence, obtained through measurements of CRP-specific antibody reactivity of crude cell extracts, indicates that the level of R195 CRP in cells containing pJH8crpR195 is lower by a factor of 4–8 than the level of 91 CRP in cells containing pKC30crp91-37 grown under identical conditions (data not shown). This suggests that the strength of the CRP* phenotype is determined, at least in part, by the cellular concentration of CRP*; a conclusion consistent with the observed concentration-dependent activation of lacP by different forms of CRP* in vitro (Ref. 19, Table III).

Detailed structural information is available for the CRP-cAMP complex form of CRP. Similar detail is lacking for the unliganded CRP structure. The results of chemical probes of protein structure and physical measurement of protein radii (see Ref. 1 for a comprehensive listing) clearly show that these forms of CRP differ. Computer analysis of CRP secondary structure based on primary amino acid sequence has led Kypr and Mrazek (38) to conclude that the amino-proximal cyclic nucleotide binding domain of CRP undergoes substantial transition upon binding cAMP. They predict that this transition involves the reorientation of a predominantly α-helix structure to the β-roll structure that characterizes the cyclic nucleotide binding pocket in CAMP-CARP crystals. This structural rearrangement appears to increase the apparent affinity of CRP for cAMP. Observations consistent with this prediction come from two sets of experiments. First, all CRP* forms tested share, in the absence of cAMP, a conformational characteristic of the wild-type CRP-cAMP complex that is protease sensitive. This is not a characteristic of uncomplexed, wild-type CRP. Second, both the 91 and R195 forms of CRP contain amino acid substitutions located outside of the CAMP binding pocket (Fig. 3) and both exhibit high apparent affinity for CAMP (Table III). This increased apparent affinity is not explained on the basis of direct mutational alteration of protein/cAMP contact sites. Similarly, Aiba et al. (17) have identified and characterized additional CRP* mutants (R148, N141, H053, and C142) that contain amino acid substitutions outside of the CAMP binding pocket. A common feature among these forms of CRP is a decreased CAMP concentration requirement for CAMP-mediated lacP activation in vivo relative to that observed for cells that contain wild-type CRP (17). These observations suggest that these mutant forms of CRP have increased affinity for CAMP.

Two forms of CRP* (220 and 222 CRP*) have been described that exhibit affinity for CAMP comparable to that of wild-type CRP yet exhibit protease sensitivity similar to other CRP* forms of CRP and to the wild-type CRP-CAMP complex (19). This might argue against a correlation between CRP conformation and affinity for cyclic nucleotide; however, both the 220 and 222 forms of CRP have isoleucine substituted for threonine at position 127. Analysis of the CRP-CAMP crystals has led to the proposal that threonine 127 hydrogen bonds with the N-6 amino group of cAMP (Ref. 39 and Fig. 3). It is likely that the 220 and 222 forms of CRP have lower apparent affinity for CAMP relative to the 91 or R195 forms due to the elimination of this putative protein/effectector contact, which we expect would decrease the stability of this interaction. Along these lines, Aiba et al. (17) have described a CRP* form of CRP (F062) that contains phenylalanine substituted for serine at position 62. The apparent affinity

Table III summarizes the properties of CRP* mutants presented in (19) and in this paper. The 91 CRP and R195 CRP show similar properties in vitro with respect to lac transcription reactions containing wild-type CRP require 1 μM cAMP to half-maximally activate lacP (19). The data presented in Fig. 2B demonstrate that R195 CRP has relatively high apparent affinity for cAMP; approximately half-maximal activation of lacP was observed at a CAMP concentration of 20 nM. In contrast, R195 CRP has lower apparent affinity for cGMP; half-maximal lacP activation required the addition of cGMP to approximately 10 μM final concentration.

**DISCUSSION**

Table III summarizes the properties of CRP* mutants presented in (19) and in this paper. The 91 CRP and R195 CRP show similar properties in vitro with respect to lac transcription in the absence or presence of cyclic nucleotides yet differ in their ability to promote cAMP-independent β-galactosidase synthesis in vivo. Cells containing the R195 CRP have a weak CRP* phenotype (Tables I and II) while cells containing the 91 CRP exhibit an intermediate CRP* phenotype (19). Semi-quantitative evidence, obtained through measurements of CRP-specific antibody reactivity of crude cell extracts, indicates that the level of R195 CRP in cells containing pJH8crpR195 is lower by a factor of 4–8 than the level of 91 CRP in cells containing pKC30crp91-37 grown under identical conditions (data not shown). This suggests that the strength of the CRP* phenotype is determined, at least in part, by the cellular concentration of CRP*, a conclusion consistent with the observed concentration-dependent activation of lacP by four forms of CRP* in vitro (Ref. 19, Table III).

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for cAMP of this form of CRP* is similar to that of the wild-type protein. CRP serine 62 is in close proximity to the adenine moiety of cAMP in CRP-cAMP complex crystals (Ref. 40 and Fig. 3B). Computer modeling places the aromatic ring of phenylalanine at position 62 inside the cyclic nucleotide binding pocket; this may hinder hydrogen bond formation between threonine 127 and N-6 cAMP and account for the lower affinity of P062 for cAMP relative to other forms of CRP.² These considerations, along with the observation that no CRP* has been described having relatively high affinity for cGMP, which cannot hydrogen bond with threonine 217 in a manner similar to cAMP, suggest that 1) one consequence of CRP* mutations is rearrangement of the amino-proximal domain of CRP such that the cAMP binding domain approximates that observed in CRP-cAMP crystals, and 2) CRP conformation plays a role in determining the affinity of the protein for cyclic nucleotide. We feel that a proposal describing a specific mechanism by which CRP* mutations mediate these effects is premature in the absence of additional information regarding the structure of unliganded CRP.

Strain 222 was derived from strain 220 by a spontaneous mutation resulting in the substitution of arginine for leucine at position 195. A primary goal of the current study was to arrive at an understanding of the mechanism by which the mutation at position 195 leads to an apparent 10-fold increase in the affinity of 222 CRP for lacP DNA compared to 220 CRP. It was anticipated that the isolated mutation might confer higher affinity for DNA compared to the wild-type protein without affecting the cyclic nucleotide requirement of the protein. Contrary to this prediction, the substitution of arginine for leucine at position 195 in CRP results in a form of CRP having altered conformation, effector specificity, requirements for promoting lacP activity in vitro and increased affinity for cAMP compared to wild-type CRP. An important conclusion to be drawn from this study is that the effects of multiple mutations in CRP can be both cumulative and interactive.

Position 195 is carboxyl-proximal to the DNA sequence recognition helix (F-helix, Ref. 39) of CRP as illustrated in Fig. 3A. R195 CRP contains the most carboxyl-proximal single amino acid substitution identified to date that confers cAMP-independent function on CRP. Another mutant allele, crpP195, substitutes proline for leucine at position 195 (see "Experimental Procedures"). Unlike R195 CRP, P195 CRP does not confer a CRP* phenotype on strain CA8445:pRK248. Cells containing P195 CRP failed to ferment any of the sugars listed in Table II in the presence or absence of cAMP. We interpret this as indicating that P195 CRP is an inactive form of the protein. Clearly, different amino acid substitutions in CRP in the carboxyl-terminal portion of the protein, specifically at position 195, can affect CRP function and response to cAMP; however, not all such substitutions confer a CRP* phenotype.

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