

## Cra-Dependent Transcriptional Activation of the *icd* Gene of *Escherichia coli*

JEAN-FRANÇOIS PROST,<sup>1</sup> DIDIER NÈGRE,<sup>1</sup> CHRISTELLE OUDOT,<sup>1</sup> KATSUHIKO MURAKAMI,<sup>2</sup>  
AKIRA ISHIHAMA,<sup>2</sup> ALAIN J. COZZONE,<sup>1\*</sup> AND JEAN-CLAUDE CORTAY<sup>1</sup>

*Institut de Biologie et Chimie des Protéines, Centre National de la Recherche Scientifique, Lyon, France,<sup>1</sup> and  
National Institute of Genetics, Mishima, Shizuoka 411, Japan<sup>2</sup>*

Received 29 June 1998/Accepted 20 November 1998

**The *icd* gene of *Escherichia coli*, encoding isocitrate dehydrogenase, was shown to be expressed from two different promoters: the previously identified *icd* P1 and a newly detected second promoter, *icd* P2, whose expression is positively regulated by the catabolite repressor-activator protein Cra, formerly called FruR. In each case, we determined the mRNA start site by primer extension analysis of *in vivo* transcripts and examined the interaction of the *icd* control region with either RNA polymerase or Cra. We observed that (i) the Cra factor binds to and activates transcription from a site centered at position  $-76.5$  within the *icd* P2 promoter region and (ii) three particular mutations in the C-terminal end of the  $\alpha$  subunit of RNA polymerase (L262A, R265A, and N268A) considerably diminish transcription initiating from the *icd* P2 promoter, as shown by *in vitro* experiments performed in the presence of mutant RNA polymerases carrying Ala substitutions.**

In microorganisms growing on acetate as the sole carbon source, isocitrate can be catabolized either by the Krebs cycle or by the glyoxylate bypass (18). In *Escherichia coli*, this branch point is regulated by the reversible phosphorylation and the concomitant inactivation of the NADP<sup>+</sup>-dependent isocitrate dehydrogenase (IDH; EC 1.1.1.42) (9, 19, 26). Such a phosphorylation or dephosphorylation process plays a pivotal role in cell survival by connecting, via the glyoxylate pathway, the metabolism of two-carbon compounds with gluconeogenesis and by allowing bacteria to draw energy through the oxidative decarboxylation steps of the Krebs cycle.

In addition to posttranslational control, IDH is also regulated at the level of expression of its gene (*icd*). It has been demonstrated that under anaerobic cell growth conditions, the *icd* gene is negatively controlled by both the ArcAB system and the Fnr factor (15). Recently, Chao et al. (5) provided direct evidence of the negative role played by ArcA in *icd* gene regulation by demonstrating *in vitro* binding of this repressor at a target operator site overlapping the *icd* promoter. Also, previous work has shown that the Cra protein (initially called FruR) exerts positive control over a number of genes and operons encoding biosynthetic and oxidative enzymes, including *icd* (reviewed by Saier and Chin [30]). In the latter case, a single Cra-binding site was detected by electrophoretic mobility shift analysis (EMSA) in the DNA fragment encompassing the regulatory region of the *icd* gene (28).

This paper focuses on the role of protein Cra in transcription of the *icd* gene. Using an *in vitro* transcription approach, we found that in addition to its main promoter (P1), the *icd* control region contains a second promoter whose activation is dependent on the action of Cra (P2). The start points relative to these promoter sites were mapped by primer extension analysis, and then the precise contacts between RNA polymerase (RNAP) and its promoters and between Cra and its DNA operator were analyzed by the base removal method and the DNase I footprinting technique, respectively. Finally, testing of

specific point mutations in the  $\alpha$  subunit of RNAP revealed that a number of RNAP-DNA contacts play a key role in transcription of the *icd* gene.

### MATERIALS AND METHODS

**Proteins.** Recombinant active Cra protein with a His<sub>6</sub> tag at its C-terminal end was prepared from *E. coli* BL21(DE3) cells (33) harboring the overproducing plasmid pJCD2 (6). Wild-type and mutant  $\alpha$  subunits of RNAP were reconstituted *in vitro* from separately purified subunits as described previously (14). The specific activity of wild-type and mutant  $\alpha$ -subunit holoenzymes was determined by measuring the level of poly(dA-dT)-dependent poly(AU) synthesis.

**Plasmids.** (i) **Plasmid pJFC2.** The *icd* promoter region (EMBL accession no. J02799) (34) was generated by PCR amplification from *E. coli* K-12 chromosomal DNA. The synthetic oligonucleotide primers used in this reaction were P<sub>*icd-EcoRI*</sub> (5'-TATGAATTCAGGTTTACGCC-3') and P<sub>*icd-PstI*</sub> (5'-TATCTGCAGGGTGATCTT-3'). Following PCR, the fragment was restricted with *EcoRI-PstI* and cloned in fusion with the *lacZ* gene into the compatible sites of the vector pNM481 (21) to create plasmid pJFC2. Plasmid pJFC2 was also used to generate radioactively labeled DNA fragments for gel retardation, base removal interference, and DNase I footprinting studies. In all cases, after restriction by *EcoRI-PstI*, the DNA fragment carrying the *icd* promoter fragment was end labeled at the *EcoRI* site by [ $\alpha$ -<sup>32</sup>P]dATP (3,000 Ci/mmol) (bottom strand), using the Klenow fragment of DNA polymerase.

(ii) **Plasmid pJFC1.** pJFC1, derived from the pUC19 vector (GenBank accession no. X02514), was used as the template for *in vitro* transcription studies. It contained the *EcoRI-PstI icd* promoter-bearing DNA fragment inserted into the corresponding sites of pUC19, upstream of the transcriptional termination signal T1T2 of the *rrnB* operon (1).

**Primer extension analysis.** RNA was isolated from *E. coli* [pJFC2] cells essentially as described by Reddy et al. (29). The oligonucleotide primer 5'-TGCATATGCGTTTTGCGTCTGCGATACGGA-3' (250 pmol) was end labeled according to the standard procedure (31), using 30  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P]ATP (3,000 Ci/mmol) and T4 polynucleotide kinase (Promega Corp.). Primer extension reaction was performed as described elsewhere (25), using 45  $\mu$ g of total cellular RNA. Extension products were resolved by electrophoresis in a 6% (wt/vol) polyacrylamide-7 M urea gel and visualized by autoradiography.

***In vitro* transcription assay.** Single-round *in vitro* transcription experiments were performed with template plasmid pJFC1 as follows. Five picomoles of supercoiled plasmid pJFC1 was preincubated for 25 min at 30°C with 1 pmol of either wild-type or mutant  $\alpha$ -subunit RNAPs in a 20- $\mu$ l assay mixture containing 50 mM Tris-acetate (pH 8.0), 100 mM potassium acetate, 8% (vol/vol) glycerol, 0.1 mM EDTA, 8 mM magnesium acetate, 0.1 mM dithiothreitol, and 500 U of RNAsin per ml. When required, 25 pmol of active Cra protein was added. Transcription reactions were initiated by the addition of 0.2 mM each ATP, GTP, and CTP, 0.01 mM UTP, 2  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]UTP (800 Ci/mmol), and 100  $\mu$ g of heparin per ml. After 10 min of incubation at 37°C, the reactions were blocked with 1 volume of gel loading buffer, the mixtures were heated at 65°C for 3 min and analyzed in a 6% polyacrylamide-7 M urea gels. Quantification of bands on the gel was achieved by densitometric analysis of the autoradiograms.

\* Corresponding author. Mailing address: Institut de Biologie et Chimie des Protéines, Centre National de la Recherche Scientifique, 7 Passage du Vercors, 69367 Lyon Cedex 07, France. Phone: 33 (0) 4.72.72.26.75. Fax: 33 (0) 4.72.72.26.01. E-mail: aj.cozzone@ibcp.fr.

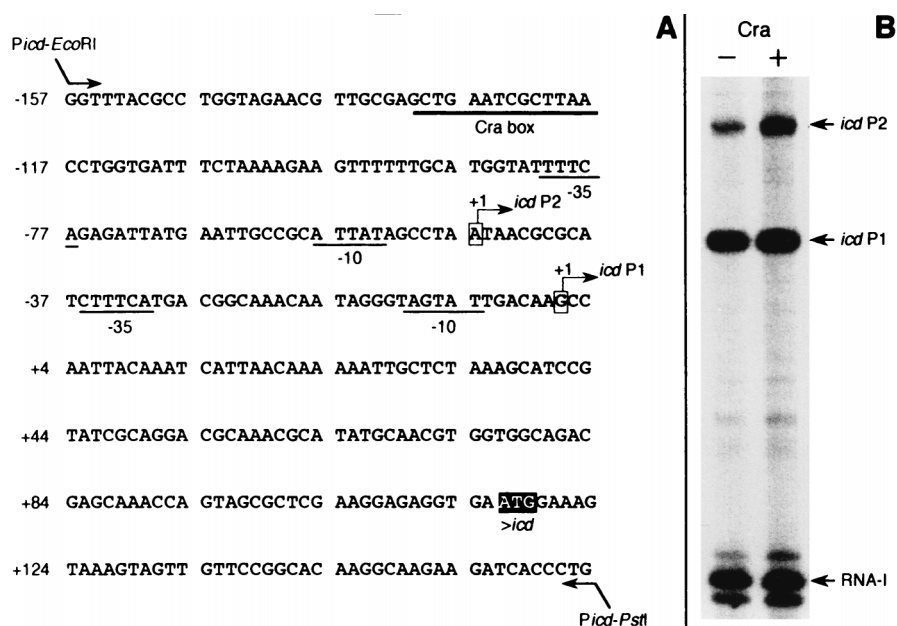


FIG. 1. (A) Nucleotide sequence of the *icd* regulatory region (from EMBL accession no. J02799). The  $-10$  and  $-35$  regions of the *icd* P1 and *icd* P2 promoters are underlined; locations of the transcription initiation sites of the two promoters are indicated by arrows. The high-affinity Cra-binding site (Cra box) is centered at position  $-76.5$  relative to the P2 transcription initiation site. (B) In vitro transcription from *icd* promoters with wild-type RNAP. Transcription assays were performed with plasmid pJFC1 as the template. The positions of the RNA-I (nt 107 to 108), *icd* P1 (nt 225), and *icd* P2 (nt 272) transcripts are shown by arrows.

**EMSA.** Gel shift assays were performed as previously described (10). Typically, a 5'-end-labeled DNA fragment ( $10^5$  cpm) was incubated with either RNAP (100 nM) or active Cra protein (50 nM) for 10 min at 25°C in 20  $\mu$ l of DNA binding buffer [12 mM HEPES-NaOH (pH 7.9), 4 mM Tris-HCl (pH 7.9), 95 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 9% [vol/vol] glycerol, 0.02% [vol/vol] Nonidet P-40, 2  $\mu$ g of poly(dI-dC) · poly(dI-dC), 10  $\mu$ g of bovine serum albumin per ml]. Complexes were resolved from free DNA on a 4% nondenaturing polyacrylamide gel and electrophoresed in Tris-glycine buffer (50 mM Tris, 384 mM glycine, 2.1 mM EDTA) at 200 V for 1 to 2 h at 25°C. Radioactive bands were visualized by autoradiography.

**DNase I footprinting.** DNase I footprinting was performed in 100  $\mu$ l of DNA binding buffer (see above) with purified Cra protein (10 or 50 nM) and the 5'-end-labeled DNA fragment ( $10^5$  cpm) that contained the *icd* promoter. Following incubation of the reaction mixture for 10 min at 25°C, 1.6 U of DNase I (Stratagene) in the presence of 2 mM  $\text{CaCl}_2$  and 6 mM  $\text{MgCl}_2$  was added; digestion was allowed to proceed for 50 s and was stopped by the addition of 10 mM EDTA. After precipitation with ethanol, nucleic acids were dissolved in formamide dye mix and heated at 80°C for 3 min prior to electrophoresis in a 6% acrylamide-7 M urea sequencing gel.

**Base removal experiments.** Base removal experiments were performed by the procedure described by Brunelle and Schleif (2) as modified by Nègre et al. (23).

## RESULTS AND DISCUSSION

**In vitro transcription of the *icd* gene.** An in vitro transcription assay was carried out with the supercoiled plasmid pJFC1, which contains the *icd* regulatory region (Fig. 1A) cloned upstream of the strong *rrnB* T1 and T2 terminators (24). The products obtained from single-round transcription experiments were analyzed by electrophoresis in a 6% sequencing gel and detected by autoradiography.

When RNAP alone was present in the reaction mixture, plasmid pJFC1 produced two major transcripts (Fig. 1B); one corresponded to RNA-I, and the other represented an RNA product comparable in length (225 bp) to the predicted transcript from the relevant P1 promoter (5). Interestingly, a third transcript (Fig. 1B, *icd* P2) about 272 bp in length could be detected as a faint band on the autoradiogram. Addition of the Cra protein resulted in an about 3.5-fold stimulation of P2 transcript synthesis, whereas no Cra-mediated stimulation of

transcription was observed at the P1 promoter under the same conditions of incubation.

Together, these data showed that besides the previously identified P1 promoter, the *E. coli icd* gene was expressed from an additional promoter, P2, whose expression was subject to Cra control.

**Location of the *icd* mRNA start sites.** Primer extension analysis was performed to identify the transcriptional start points of in vivo transcription from *icd* P1 and *icd* P2. Total cellular RNA was primed with a radiolabeled oligonucleotide complementary to the *icd* regulatory sequence, and this primer was extended by using reverse transcriptase. The primer used for cDNA synthesis was also used in a DNA sequencing reaction to determine the length of each extension DNA product.

To identify the initiation sites of the plasmid-encoded *icd* gene promoters, RNA was isolated from *E. coli* JM109[pJFC2] and mapped by as the primer a 30-mer synthetic oligodeoxyribonucleotide complementary to nucleotides (nt) +40 to +69 of the *icd* gene 5' flanking sequence (Fig. 1A). In agreement with the in vitro transcription data described above, the results presented in Fig. 2 indicate that in vivo transcription of the *icd* gene was initiated at two sites, even though the corresponding extension products differed greatly in intensity. This analysis thus confirmed both the position of the start site at P1 (designated *icd* P1 in Fig. 1A) that had been recently reported (5) and the occurrence of a new initiation site at P2 (designated *icd* P2 in Fig. 1A) which could be precisely mapped at an adenosine residue located 162 bp upstream from the translation initiation codon.

**Interaction of RNAP with the *icd* promoter region.** In bacteria, the transcription process initiates through the formation of a closed complex between RNAP and the promoter site, followed by the isomerization to a transcription-competent open structure in which the DNA encompassing residues from approximately  $-12$  to  $+2$  (with respect to the transcription start site at  $+1$ ) represents the melting domain. To assess the

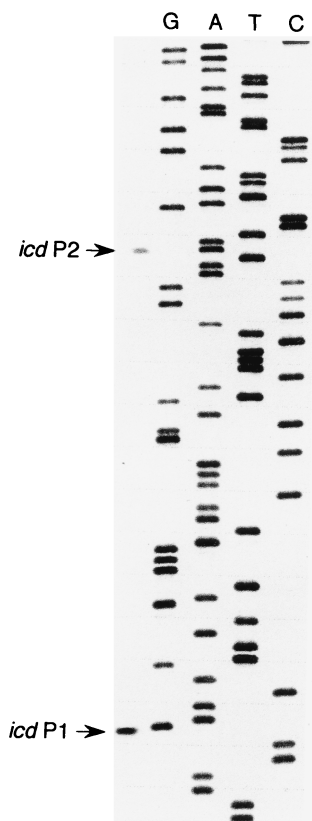


FIG. 2. Mapping of the *icd* transcriptional start sites by primer extension analysis. A  $^{32}\text{P}$ -end-labeled oligonucleotide primer ( $10^6$  cpm) was annealed to 45  $\mu\text{g}$  of total RNA isolated from *E. coli* JM109[pJCD2] cells and extended by using deoxyribonucleoside triphosphates and avian myeloblastosis virus reverse transcriptase. The cDNA products were separated by electrophoresis on a 6% polyacrylamide-7 M urea gel. The mobility of cDNA products was compared to a DNA sequencing ladder (GATC) generated with the same primer as that used for the primer extension reactions. Arrows indicate the nucleotides corresponding to the lengths of the extension products.

role of individual base residues in the specific interaction between RNAP and the promoter, the base removal interference technique (2) has been found to be a powerful approach (17, 23, 36).

The *EcoRI*-*PstI* end-labeled DNA fragment (328 bp) containing the *icd* promoter region (Fig. 1A) was subjected to partial base-specific cleavage by the method of Maxam and Gilbert (20) and incubated with  $\sigma^{70}$ -saturated RNAP holoenzyme (24). After separation by gel shift electrophoresis in a preparative 4% nondenaturing polyacrylamide gel and piperidine treatment, free and complexed DNA molecules were analyzed in a 6% sequencing gel. The corresponding electrophoretic pattern (Fig. 3) demonstrates that RNAP binding was greatly enhanced when gaps were generated in the *icd* regulatory sequence between nt -4 and -11 and between -52 and -60. The particular areas thus revealed by the base removal technique could be defined as the melted domains that are integral parts of the open complexes formed upon initiation of transcription at P1 and P2 (3, 32). Taken together, our data showed that the matches to the consensus sequences in the -10 (TATAAT) and -35 (TTGACA) regions (12, 13) of the two promoters were four of six (TAGTAT) and three of six (CTTCA) for *icd* P1, as already reported (5), and four of six for the -10 sequence (CATTAT) and three of six for the putative -35 hexamer (CTTCA) in the *icd* P2 region (Fig.

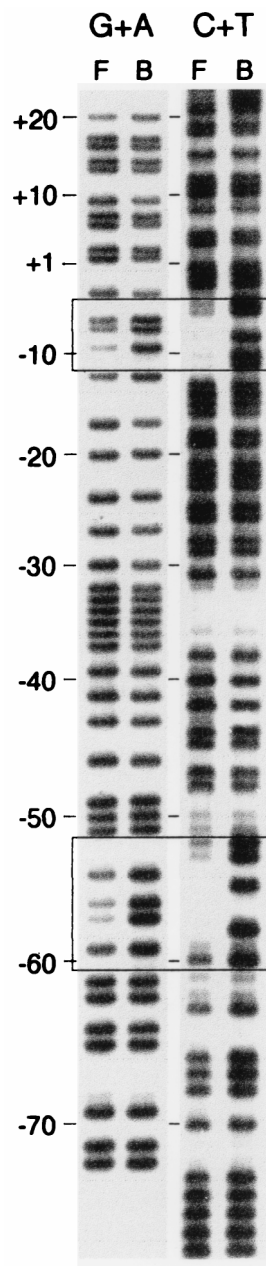


FIG. 3. Identification of RNAP-*icd* DNA contacts by base removal footprinting. The 328-bp *icd* regulatory region,  $^{32}\text{P}$ -labeled at the *EcoRI* site (Fig. 1A), was treated with either formic acid to remove G+A or hydrazine to remove C+T and then incubated with RNAP (approximately 10 nM active protein) prior electrophoresis on a 4% preparative polyacrylamide gel at high ionic strength. Free and complexed DNA molecules were then subjected to piperidine cleavage and analyzed on a 6% sequencing gel to reveal the positions of the interfering modifications. Lanes: B, modified DNA isolated from complexes; F, DNA that had dissociated or was free of complex.

1A). The *in vitro* transcription assays performed in the absence of the Cra protein demonstrated that *icd* P1 is stronger than *icd* P2, which suggests that the way in which RNAP recognizes *icd* P2 differs from that used for *icd* P1 (Fig. 1B). Moreover, it appeared that the formation of the initiation complex at *icd* P2 was activated in the presence of Cra, whose main function would be to bend the DNA region of the promoter (25) (see below).



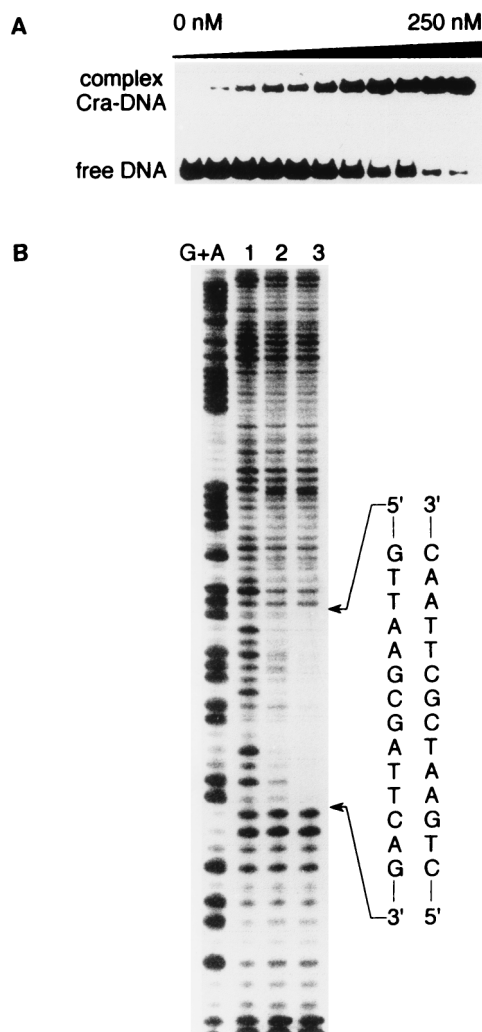


FIG. 4. Characterization of the Cra-*icd* regulatory region complex. (A) Relative strength of binding of Cra to the *icd* regulatory region. The 328-bp  $^{32}\text{P}$ -labeled *icd* DNA (4 pM) was incubated at 25°C with increasing (from 0 to 250 nM) concentrations of Cra protein and analyzed by EMSA. (B) Cra-mediated protection of the *icd* regulatory region against digestion by DNase I. Target DNA was incubated in the absence (lane 1) or in the presence of 10 and 50 nM Cra protein (lanes 2 and 3, respectively). Samples were separated on a 6% polyacrylamide sequencing gel. G+A shows the Maxam-Gilbert sequencing ladder of the probe; arrows delineate the footprint produced by Cra.

**Cra binding to the *icd* promoter region.** The binding affinity of the active Cra factor for the *icd* promoter region was measured by the EMSA (10). The radiolabeled 328-bp DNA fragment carrying both *icd* P1 and *icd* P2 was incubated with increasing concentrations of Cra, and the corresponding complexes were resolved from free DNA by gel electrophoresis and quantified after autoradiography. At the concentrations of Cra used in these experiments, single stable complexes could be resolved by EMSA (Fig. 4A), which indicated that Cra could interact in a specific manner with one operator site in the *icd* regulatory region. The bandshift assay was performed at a low concentration of DNA (4 pM) such that the approximate free protein concentration equaled the total protein concentration; thus, at half-maximal saturation, there was >10-fold molar excess of protein relative to nucleic acid (4). The equilibrium binding constant,  $K_d$ , could therefore be derived as the total protein concentration at half-maximal saturation. The  $K_d$  value

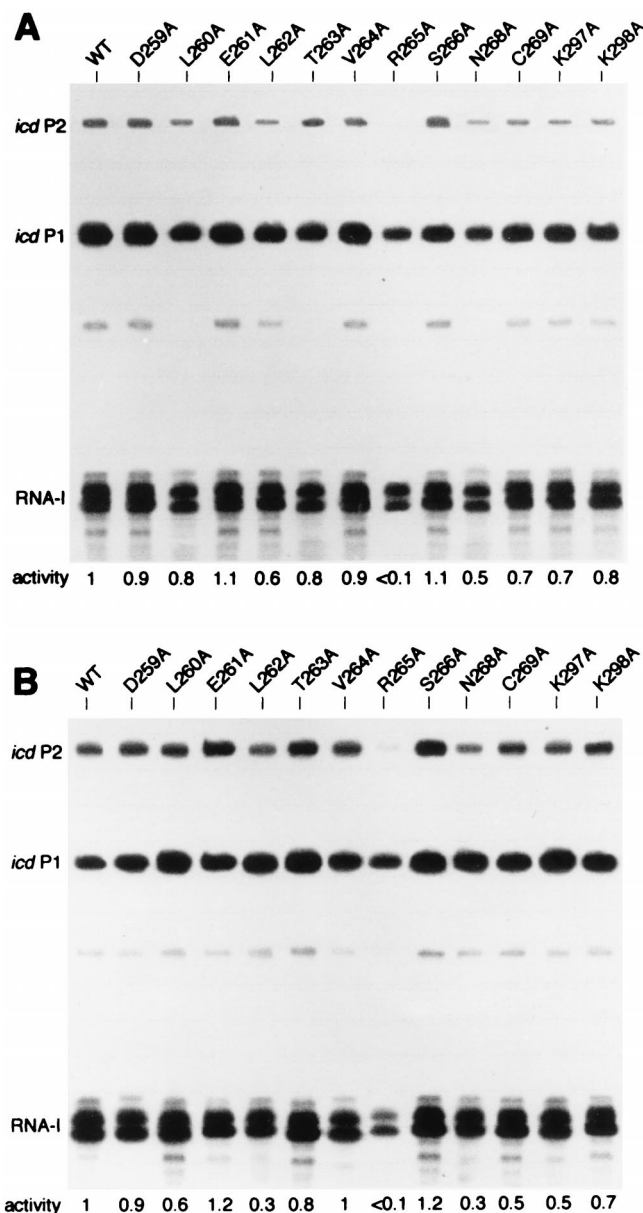


FIG. 5. In vitro transcription by wild-type (WT) and mutant RNAPs. Reconstituted RNAP holoenzymes containing Ala substitutions in the  $\alpha$ CTD were assayed for transcription in the presence (A) or absence (B) of Cra. Single-round transcriptions were performed by using supercoiled plasmid pJFC1 as the template in the presence of mutant RNAP and protein Cra in a molar ratio of 1:1. Transcripts were separated in a 6% sequencing gel and detected by autoradiography. The ratio of the transcriptional activity at *icd* P2 relative to that at *icd* P1 is indicated below each gel.

for Cra binding to the *icd* regulatory region at pH 7.9 and 25°C was about 10 nM, which indicated a relatively high binding affinity of Cra for this particular region of DNA.

To characterize further the zone of the *icd* P2 promoter that specifically interacts with Cra, the nucleotide sequence protected from DNase I digestion after binding of the protein was analyzed. The results presented in Fig. 4B show that upon addition of Cra, a protected region centered at position -76.5 with respect to the transcription start site of the downstream P2 promoter could be identified. This protected region contained the sequence 5'-CTGAATC/GCTTAAC-3', which is

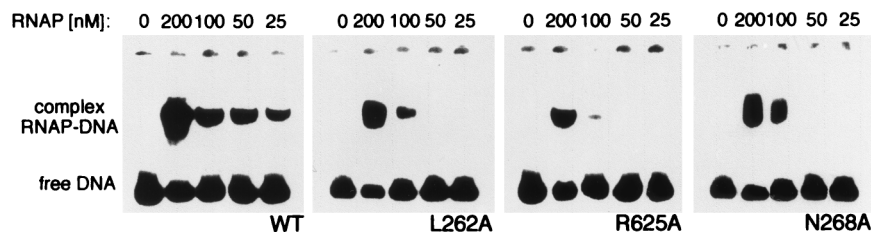


FIG. 6. Characterization of the complexes between wild-type (WT) or mutant RNAPs and the *icd* P2 promoter. A 120-bp  $^{32}\text{P}$ -labeled DNA carrying the *icd* P2 promoter region was incubated at 25°C with a decreasing concentration of RNAP and analyzed by EMSA. Positions of the protein-DNA complex and free DNA are indicated.

displaced by one base pair compared to the previously determined (23, 27, 28) consensus Cra binding site sequence, 5'-GCTGAATC/GCT-3'. This finding suggested that similarly to the *ppsA* promoter (25, 27), transcription is activated only when Cra and RNAP bind to opposite faces of the DNA helix. In this regard, previous reports on the regulation of different transcription systems have demonstrated that when a correct helical phasing with RNAP is achieved, the cyclic AMP receptor protein, for instance, is able to activate transcription at a varying distance upstream from the transcription start site of the promoter, even though the extent of such activation varies with the length of the spacer (7, 11, 35, 37). It has been proposed that upon binding to DNA, Cra, like the cyclic AMP receptor protein, helps to recruit RNAP by bending the proximal DNA region located upstream of the promoter, thereby favoring tight contacts between DNA and the C-terminal domain of the  $\alpha$  subunit ( $\alpha\text{CTD}$ ) of RNAP (7, 25).

**Effects of Ala substitutions in the  $\alpha\text{CTD}$  of RNAP on *icd* transcriptional activation.** To determine the importance of the  $\alpha\text{CTD}$  in *icd* P2 transcription, we treated a series of alanine mutant RNAPs containing point mutations in this region (22) for transcription in the presence of plasmid pJFC1. The results (Fig. 5) showed that each mutant RNAP tested could initiate transcription from the *icd* P1 promoter at nearly the same rate as the wild-type enzyme. On the other hand, the transcription initiated from the *icd* P2 promoter, with or without Cra, was considerably reduced when incubation was carried out with mutant RNAPs containing substitution L262A, R265A, and N268A ( $\alpha[\text{L}262\text{A}]$ ,  $\alpha[\text{R}265\text{A}]$ , and  $\alpha[\text{N}268\text{A}]$ ), whereas a less drastic effect was observed with mutant RNAPs  $\alpha[\text{L}260\text{A}]$ ,  $\alpha[\text{C}269\text{A}]$ , and  $\alpha[\text{K}297\text{A}]$ . Moreover, comparison of the *icd* P2/*icd* P1 transcription ratio, in both the presence and absence of Cra, showed that both basal transcription (Fig. 5A) and the Cra-dependent activation of transcription (Fig. 5B) were affected concomitantly by these mutations. This result strongly supports the hypothesis that during formation of the closed complex, basal transcription initiation as well as Cra-dependent activation of transcription strictly rely on contacts established between the  $\alpha$  subunit and the proximal DNA region upstream from the promoter.

We assessed the DNA-binding capacity of each of the three mutants ( $\alpha[\text{L}262\text{A}]$ ,  $\alpha[\text{R}265\text{A}]$ , and  $\alpha[\text{N}268\text{A}]$ ) that exhibited the highest deficiency in transcription initiation. In each case, EMSA in the presence of the radiolabeled *icd* P2 promoter was performed. The results (Fig. 6) showed that DNA binding was strongly impaired in all three mutant enzymes compared to the wild-type RNAP. It therefore appears that any substitution of an amino acid residue either located on the DNA contact surface of the  $\alpha$  subunit (Arg-265 and Asn-268) or required for maintaining the correct conformation of this DNA-binding surface (Leu-262) (16) would result in the destabilization of

the  $\alpha$ -subunit-mediated anchoring of the RNAP on the *icd* P2 promoter.

Concerning the suggested role of Cra in the activation of transcription at *icd* P2, the DNA bending caused by this protein (25) at its binding site around position  $-76.5$  would modify the structure of the DNA region upstream of the promoter so as to facilitate its interaction with RNAP and thereby activate formation of the closed complex. Cra would thus compensate for the absence of an UP (upstream) element in *icd* P2 (8) by changing the structure of proximal DNA so as to make it operate like an UP module (22).

#### ACKNOWLEDGMENTS

This work was supported by the CNRS (UPR 412), Université de Lyon, Institut Universitaire de France, and grants from the Ministry of Education, Science and Culture of Japan.

We thank Antony W. Coleman for reading the manuscript. We also thank Christian Van Herrewwege and Alain Bosch for help in iconography.

#### REFERENCES

- Brownlee, G. G., F. Sanger, and B. G. Barrell. 1967. Nucleotide sequence of 5S ribosomal RNA from *Escherichia coli*. *Nature* **215**:735-736.
- Brunelle, A., and R. F. Schleif. 1987. Missing contact probing of DNA-protein interactions. *Proc. Natl. Acad. Sci. USA* **84**:6673-6676.
- Buc, H., and W. R. McClure. 1985. Kinetics of open complex formation between *Escherichia coli* RNA polymerase and the lac UV5 promoter. Evidence for a sequential mechanism involving three steps. *Biochemistry* **24**:2712-2723.
- Carey, J. 1988. Gel retardation at low pH resolves trp repressor-DNA complexes for quantitative study. *Proc. Natl. Acad. Sci. USA* **85**:975-979.
- Chao, G. C., J. Shen, C. P. Tseng, S. J. Park, and R. P. Gunsalus. 1997. Aerobic regulation of isocitrate dehydrogenase gene (*icd*) expression in *Escherichia coli* by the *arcA* and *fur* gene products. *J. Bacteriol.* **179**:4299-4304.
- Cortay, J. C., D. Nègre, M. Scarabel, T. M. Ramseier, N. B. Vartak, J. Reizer, and A. J. Cozzone. 1994. *In vitro* asymmetric binding of the pleiotropic regulatory protein, FruR, to the *ace* operator controlling glyoxylate shunt enzyme synthesis. *J. Biol. Chem.* **269**:14885-14891.
- Dethiollaz, S., P. Eichenberger, and J. Geiselmann. 1996. Influence of DNA geometry on transcriptional activation in *Escherichia coli*. *EMBO J.* **15**:5449-5458.
- Estrem, S. T., T. Gaal, W. Ross, and R. L. Gourse. 1998. Identification of an UP element consensus sequence for bacterial promoters. *Proc. Natl. Acad. Sci. USA* **95**:9761-9766.
- Garnak, M., and H. C. Reeves. 1979. Purification and properties of phosphorylated isocitrate dehydrogenase of *Escherichia coli*. *J. Biol. Chem.* **254**:7915-7920.
- Garner, M. M., and A. Revzin. 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of the *Escherichia coli* lactose operon regulatory system. *Nucleic Acids Res.* **9**:3047-3060.
- Gaston, K., A. Bell, A. Kolb, H. Buc, and S. Busby. 1990. Stringent spacing requirements for transcription activation by CRP. *Cell* **62**:733-743.
- Harley, C. B., and R. P. Reynolds. 1987. Analysis of *E. coli* promoter sequences. *Nucleic Acids Res.* **15**:2343-2346.
- Hawley, D. K., and W. R. McClure. 1980. *In vitro* comparison of initiation properties of bacteriophage lambda wild-type PR and  $\times 3$  mutant promoters.

- Proc. Natl. Acad. Sci. USA **77**:6381–6385.
14. **Igarashi, K., and A. Ishihama.** 1991. Bipartite functional map of the *E. coli* RNA polymerase alpha subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. *Cell* **32**:319–325.
  15. **Iuchi, S., and L. Weiner.** 1996. Cellular and molecular physiology of *Escherichia coli* in the adaptation to aerobic environments. *J. Biochem.* **120**:1055–1063.
  16. **Jeon, Y. H., T. Negishi, M. Shirakawa, T. Yamazaki, N. Fujita, A. Ishihama, and Y. Kyogoku.** 1995. Solution structure of the activator contact domain of the RNA polymerase  $\alpha$  subunit. *Science* **270**:1495–1497.
  17. **Jorgensen, E. D., R. K. Durbin, S. S. Risman, and W. T. McAllister.** 1991. Specific contacts between the bacteriophage T3, T7 and SP6 RNA polymerases and their promoters. *J. Biol. Chem.* **266**:645–651.
  18. **Kornberg, H. L.** 1966. The role and control of the glyoxylate shunt in *Escherichia coli*. *Biochem. J.* **99**:1–11.
  19. **LaPorte, D. C., and D. E. Koshland, Jr.** 1982. A protein with kinase and phosphatase activities involved in regulation of tricarboxylic acid cycle. *Nature* **300**:458–460.
  20. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**:499–560.
  21. **Minton, N. P.** 1984. Improved plasmid vectors for the isolation of translational *lac* gene fusions. *Gene* **31**:269–273.
  22. **Murakami, K., N. Fujita, and A. Ishihama.** 1996. Transcription factor recognition surface on the RNA polymerase alpha subunit is involved in contact with the DNA enhancer element. *EMBO J.* **15**:4358–4367.
  23. **Nègre, D., C. Bonod-Bidaud, C. Geourjon, G. Deléage, A. J. Cozzone, and J. C. Cortay.** 1996. Definition of a consensus DNA-binding site for the *Escherichia coli* pleiotropic regulatory protein, FruR. *Mol. Microbiol.* **21**:257–266.
  24. **Nègre, D., C. Bonod-Bidaud, C. Oudot, J. F. Prost, A. Kolb, A. Ishihama, A. J. Cozzone, and J. C. Cortay.** 1997. DNA flexibility of the UP element is a major determinant for transcriptional activation at the *Escherichia coli* acetate promoter. *Nucleic Acids Res.* **25**:713–718.
  25. **Nègre, D., C. Oudot, J. F. Prost, K. Murakami, A. Ishihama, A. J. Cozzone, and J. C. Cortay.** 1998. FruR-mediated transcriptional activation at the *ppsA* promoter of *Escherichia coli*. *J. Mol. Biol.* **276**:355–365.
  26. **Nimmo, G. A., and H. G. Nimmo.** 1984. The regulatory properties of isocitrate dehydrogenase kinase and isocitrate dehydrogenase phosphatase from *Escherichia coli* and the role of these activities in the control of isocitrate dehydrogenase. *Eur. J. Biochem.* **141**:409–414.
  27. **Ramseier, T. M., S. Bledig, V. Michotey, R. Feghali, and M. H. Saier, Jr.** 1995. The global regulatory protein FruR modulates the direction of carbon flow in *Escherichia coli*. *Mol. Microbiol.* **16**:1157–1169.
  28. **Ramseier, T. M., D. Nègre, J. C. Cortay, M. Scarabel, A. J. Cozzone, and M. H. Saier, Jr.** 1993. *In vitro* binding of the pleiotropic transcriptional regulatory protein, FruR, to the *fru*, *pps*, *ace*, *pts* and *icd* operons of *Escherichia coli* and *Salmonella typhimurium*. *J. Mol. Biol.* **234**:28–44.
  29. **Reddy, K. J., R. Webb, and L. A. Sherman.** 1990. Bacterial RNA isolation with one-hour centrifugation in a table-top ultracentrifuge. *BioTechniques* **8**:250–251.
  30. **Saier, M. H., Jr., and A. M. Chin.** 1990. Energetics of the bacterial phosphotransferase system in sugar transport and the regulation of carbon metabolism, p. 273–299. *In* T. A. Krulwich (ed.), *Bacterial energetics*. Academic Press, Inc., New York, N.Y.
  31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  32. **Sasse-Dwight, S., and J. Gralla.** 1989. KMnO<sub>4</sub> as a probe for *lac* promoter DNA melting and mechanism *in vivo*. *J. Biol. Chem.* **264**:8074–8081.
  33. **Studier, W. F., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**:60–89.
  34. **Thorsness, P. E., and D. E. Koshland, Jr.** 1987. Inactivation of isocitrate dehydrogenase by phosphorylation is mediated by the negative charge of the phosphate. *J. Biol. Chem.* **262**:10422–10425.
  35. **Ushida, C., and H. Aiba.** 1990. Helical phase dependent action of CRP: effect of the distance between the CRP site and the –35 region on promoter activity. *Nucleic Acids Res.* **18**:6325–6330.
  36. **Werel, W., P. Schickor, and H. Heumann.** 1991. Flexibility of the DNA enhances promoter affinity of *Escherichia coli* RNA polymerase. *EMBO J.* **10**:2589–2594.
  37. **Wing, H., S. Williams, and S. Busby.** 1995. Spacing requirements for transcription activation by *Escherichia coli* FNR protein. *J. Bacteriol.* **177**:6704–6710.