The Cryptic Adenine Deaminase Gene of *Escherichia coli*

SILENCING BY THE NUCLEOID-ASSOCIATED DNA-BINDING PROTEIN, H-NS, AND ACTIVATION BY INSERTION ELEMENTS

Carsten Petersen‡§, Lisbeth Birk Møller‡, and Poul Valentim-Hansen

From the ‡Department of Biological Chemistry, Institute of Molecular Biology, University of Copenhagen, Salkogade 83H, DK1307 Copenhagen K, §John. F. Kennedy Institute, Gl. Landevej 7, 2600 Glostrup, and †Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

In *Escherichia coli* there are two pathways for conversion of adenine into guanine nucleotides, both involving the intermediary formation of IMP. The major pathway involves conversion of adenine into hypoxanthine in three steps via adenosine and inosine, with subsequent phosphoribosylation of hypoxanthine to IMP. The minor pathway involves formation of ATP, which is converted via the histidine pathway to the purine intermediate 5-amino-4-imidazolecarboxamide ribonucleotide and, subsequently, to IMP. Here we describe *E. coli* mutants, in which a third pathway for conversion of adenine to IMP has been activated. This pathway was shown to involve direct deamination of adenine to hypoxanthine by a manganese-dependent adenine deaminase encoded by a cryptic gene, *yicP*, which we propose be renamed *ade*. Insertion elements, located from −145 to +13 bp relative to the transcription start site, activated the *ade* gene as did linked mutations in the *hns* gene, encoding the histone-like protein H-NS. Gene fusion analysis indicated that *ade* transcription is repressed more than 10-fold by H-NS and that a region of 231 bp including the *ade* promoter is sufficient for this regulation. The activating insertion elements essentially eliminated the H-NS-mediated silencing, and stimulated *ade* gene expression 2–3-fold independently of the H-NS protein.

*Escherichia coli* auxotrophic mutants can utilize adenine as the sole source of purines. Conversion of adenine into guanine nucleotides occurs by two different pathways that converge on IMP and utilize the subsequent reactions of the de novo synthesis pathway to guanine nucleotides (1). The major pathway involves conversion of adenine to hypoxanthine in three steps involving the intermediate formation of adenosine and inosine (Fig. 1). The first and third reactions in this sequence are catalyzed by the *deoD* gene product, purine nucleoside phosphorylase, whereas the second step is catalyzed by adenosine deaminase encoded by the *add* gene. Hypoxanthine in turn is converted to IMP by hypoxanthine or guanine phosphoribosyltransferases encoded by the *hpt* and *gpt* genes, respectively.

The minor pathway involves formation of ATP, which is converted via the histidine pathway to the purine intermediate 5-amino-4-imidazolecarboxamide ribonucleotide and subsequently to IMP (Fig. 1). The flux through this pathway is limited because the first enzyme of the histidine pathway, HisG, is subject to strong feedback inhibition by the end product histidine (2). Thus, purine-requiring mutants in which the major pathway is blocked by mutation of the *deoD* gene only grow very slowly with adenine as the sole source of purines, and this residual growth can be eliminated by the addition of histidine to the growth medium (3). These findings indicate that there are no other pathways for converting adenine into guanine nucleotides. Specifically, studies of enzymatic activities in crude extracts indicated that *E. coli* contains no adenine deaminase activity, which might convert adenine directly to hypoxanthine by deamination (4). Nevertheless, Kocharyan et al. (5) reported the isolation of *E. coli* mutants, in which an apparently cryptic adenine deaminase gene had been activated. The genetic locus affected in these mutants, however, was not identified nor mapped.

The paradigm example of cryptic genes in *E. coli* is the *bgl* operon involved in metabolism of aromatic β-glucosides. A key element in the silencing of the *bgl* operon is the small abundant nucleoid-associated protein, H-NS, which probably forms a repressing nucleoprotein complex upon binding to silencer DNA regions flanking the *bgl* promoter (6–10). In addition to the *bgl* operon and other cryptic genes the H-NS protein also modulates the expression of a large number of active *E. coli* genes, usually by repressing transcription initiation (11, 12). H-NS binds to DNA with no obvious sequence specificity, but specific binding sites tend to be AT-rich and intrinsically bend (13–16), as also observed for the upstream silencing region of the *bgl* operon (6, 8, 17). Binding of H-NS generally induces strong condensation of DNA, and thus, the protein has been implicated in the organization and compaction of the bacterial nucleoid (18). H-NS consists of an N-terminal oligomerization domain and a C-terminal DNA binding domain, and the ability of the protein to condense DNA and repress transcription apparently depends on its ability to oligomerize (19–21).

In the present work we have isolated and characterized mutants with increased adenine deaminase activity and demonstrate that this activity is due to a manganese-dependent adenine deaminase encoded by a cryptic gene, *yicP*, which we propose be renamed *ade*. In agreement with these findings, purified YicP protein has recently been shown to possess significant adenine deaminase activity (22). To define the elements responsible for the cryptic nature of the *ade* gene, we have identified a large number of cis- and trans-acting mutations that lead to activation of gene expression. Like the *bgl* promoter, the *ade* promoter region was found to be extremely AT-rich and subject to strong repression by the H-NS protein. As also observed for the *bgl* operon, we found that insertion of a variety of IS elements within an extended region surrounding the *ade* promoter resulted in relief of the H-NS-mediated
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FIG. 1. Pathways of purine metabolism in E. coli. The adenine deaminase reaction described in this work is symbolized by an open arrow. The two previously described pathways for conversion of adenine to IMP are indicated by bold arrow. A, adenine; AR, adenosine; Hx, hypoxanthine; HxR, inosine; X, xanthine; XR, xanthosine; G, guanine; GR, guanosine; AICAR, 5-amino-4-imidazolecarboxamidine ribonucleotide.

Plasmid Constructions—DNA manipulations, transformations, and restriction analyses were performed according to standard procedures (20). PCR amplifications were performed on 1 μg of genomic DNA using Pfu polymerase (Stratagene) according to the manufacturer’s recommendations. The DNA oligonucleotides used as primers in PCR reactions are described in Table II. The sequence of cloned PCR fragments were verified by sequencing on an ABI377 DNA sequencer (Applied Biosystems/PerkinElmer Life Sciences).

For construction of pAde, the ade gene and flanking regions (nucleotides 3612–5598) were PCR-amplified from genomic DNA with the ade-R1 and ade-Bam primers (Table II) and inserted between the EcoRI and BamHI sites of the medium-copy vector pBR322 (30).

For construction of medium-copy ade-lacZ gene fusions, the ade promoter and 65 base pair of N-terminal coding region was PCR-amplified from genomic DNA and inserted between unique EcoRI and HindIII sites in the lacZ gene fusion vector pCN302, which is based on the pBR322 replicon (24). The ade promoter fragments in pCN2421 and pCN2422 were amplified with the ade-R1 and ade-H3 primers (Table II), whereas the promoter insert in pCN2534 was generated with the ade-R12 and ade-H3 primers (Table II). A low-copy derivative, pCN5155, of the IS1-activated ade-lacZ fusion, pCN2422, was constructed by inserting the ade promoter fragment into the low-copy lacZ gene fusion vector, pCN2423, which is based on the pSC101 replicon (23).

Assays of Adenine Deaminase Activity in Crude Extracts—30 ml of bacterial culture was harvested on ice at A<sub>450</sub> = 0.5. Cells were collected by centrifugation, washed, and resuspended in 50 mM Tris–HCl, pH 7.5, to A<sub>450</sub> = 20. Cells were disrupted by sonic treatment, and the extract was cleared by centrifugation at 20,000 × g for 3 min in a refrigerated microcentrifuge. Assays were performed at 37 °C by mixing appropriately diluted extract with 8.4 mM deoxycytidine (4.4 Cpm/mg) at a final concentration of 0.5 mM in a total volume of 50 μl of 40 mM Tris–HCl, pH 7.5, 5 mM MnCl<sub>2</sub>. At timed intervals, samples of 15 μl were taken out, boiled for 2 min, and cooled on ice. After a 3-min centrifugation at 20,000 × g, 5 μl of supernatant was applied to a polyethyleneimine thin layer chromatography plate. Substrate and products were separated by chromatography in water and subsequently quantitated by counting in an Instant Imager (Packard Instrument Co.). The enzymatic activities were calculated from the initial slope of a plot of the amount of radioactive substrate remaining as a function of time (see Fig. 2). The reported activities are the averages of two independent determinations, which deviated less than 10% from the average. The adenine deaminase was strongly dependent on manganese ions; the enzymatic activity decreased more than 30-fold if MnCl<sub>2</sub> was omitted from the assay (data not shown).

Measurements of β-Galactosidase Synthesis—Differential rates of

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silencing. The results suggest that these IS<sup>1</sup> elements interfered with the formation of an H-NS-DNA complex, which would otherwise sequester the adjacent ade promoter region.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Media—The bacterial strains used in this study are all derivatives of E. coli K12 and are listed in Table I. Generalized transductions with lysates of bacteriophage P1vir were performed as described (26). Minimal medium plates contained AB minimal medium (27) solidified with 2% of Difco Bacto agar and supplemented with 0.2% glucose or glycerol as the carbon source, 15 μg/ml nucleobases, 30 μg/ml nucleosides, or 40 μg/ml methionine and histidine when required. The rich medium was Luria broth (LB). Liquid cultures were grown in glucose minimal medium supplemented with 15 μg/ml hypoxanthine and 0.3% casamino acids unless otherwise indicated.

Selection of Mutants with Increased Adenine Deaminase Activity—An auxotrophic purE deoD strain, CN1980 (Table I), was plated on glucose minimal medium containing 40 μg/ml histidine and 15 μg/ml adenine as the sole source of purines. Mutants that were still auxotrophic and capable of utilizing adenine as a purine source were characterized further as described under "Results." Several independent selections were performed. To facilitate subsequent analysis of the mutants obtained, some of these selections were performed on strains that were isogenic with CN1980 except that they contained gsk mutant alleles which did not give rise to kanamycin resistance. Verification of genomic mutations by colony PCR amplification and DNA sequencing was performed as described previously (23).

Isolation of an ade::cam Disruption Mutant, CN2451—A phage λ-sensitive LamB<sup>+</sup> derivative of CN2388–2 (Table I) was mutagenized with mini-Tn10 cam from ANK1324 as described (28). After penicillin enrichment (23), we isolated chloramphenicol-resistant clones that had lost the ability to utilize adenine as a purine source by replica plating onto glucose minimal plates containing either adenine or hypoxanthine as a purine source. One of these mutants, CN2451, gave rise to a PCR product of ~1.5 kilobases with the cam-down and ade-Bam primers (Table II), and sequencing of this DNA fragment showed that the cam gene was located at positions 4156–4164 within the ade gene in the same orientation as ade.

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1 The abbreviations used are: IS, insertion elements; CRP, cAMP receptor protein.

2 The GenBank™ accession numbers for ade<sub>bic</sub>P and hns DNA sequences are AE000444 and AE000222, respectively.
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**Table I**  
Bacterial strains

| Strain<sup>a</sup> | Genotype<sup>b</sup> | Reference/Source/Construction<sup>c</sup> |
|------------------|---------------------|-----------------------------------------|
| CN1927 (NK5526)  | hisG213::Tn10 In(rrnD-rrnE)1 | N. Kleckner via CGSC<sup>d</sup> |
| CN1880           | [S0063]purE deoD gsk::kan-1 | (23) |
| CN2349           | F' araD<sup>Δ</sup>coB-lacI3 thi recA56 | (24) |
| CN2388-2         | [S0063]purE deoD gsk yicO-2::IS1 | Growth on adenine as a purine source |
| CN2388-4         | [S0063]purE deoD gsk yicO-6::IS4 | Growth on adenine as a purine source |
| CN2388-15        | [S0063]purE deoD gsk yicO-15::IS1 | Growth on adenine as a purine source |
| CN2388-16        | [S0063]purE deoD gsk hns-16::IS1 | Growth on adenine as a purine source |
| CN2451           | [CN2388-2]lamB<sup>Δ</sup> ade::cam | See “Experimental Procedures” |
| CN2470 (GM230)   | hns206::Tn10 and other markers (SupF<sup>e</sup>) | |
| CN2479           | CN1880:hns206::Tn10 | |
| CN2481           | CN2388-2::ade::cam | |
| CN2498           | CN2389::hns206::Tn10 | |
| CN2588           | CN2388-2::hns206::Tn10 | CN1880 × P1(CN2470), Tet<sup>e</sup>(SupF<sup>e</sup>) |
| CN2589           | CN2388-6::hns206::Tn10 | CN2388-2 × P1(CN2451), Cam<sup>+</sup> |
| CN2590           | CN2388-15::hns206::Tn10 | CN2388-2 × P1(CN2479), Tet<sup>e</sup> |

<sup>a</sup> Original strain designations are given in parenthesis.  
<sup>b</sup> [S0063]: F lac<sup>+</sup> metB rpsL relA spot<sup>F</sup> supE1 lamB lon<sup>+</sup>.  
<sup>c</sup> Unselected markers are shown in parenthesis. Resistance to tetracycline, chloramphenicol, or kanamycin was scored on LB medium containing 15, 20, or 25 µg/ml, respectively, of the antibiotics.  
<sup>d</sup> Coli Genetic Stock Center, Yale University, New Haven, CT.  
<sup>e</sup> hns206::Tn10 was originally designated osmZ205::Tn10.  
<sup>f</sup> Using P1(CN2470), hns206::Tn10 was transduced into CN2349, which was transiently transformed with an unstable plasmid, pMAS53, containing a functional recA gene and a temperature-sensitive replicon (25).

β-galactosidase synthesis were measured at 37 °C as described previously (31).

**RNA Isolation and Primer Extension Analysis**—Bacterial RNA was prepared by hot phenol extraction, and primer extension analysis was performed on 5 µg of total RNA as described (32). We used a 32P-labeled DNA primer complementary to nucleotides 41–50 of the lacZ coding sequence (1224, see Table II).

**RESULTS**

Selection of Mutants with Increased Conversion of Adenine to Guanine Nucleotides—Mutants with an activated adenine deaminase gene were sought by plating a purine-requiring deoD mutant strain, such as CN1980 (Table I), on glucose minimal medium containing adenine and histidine (see “Experimental Procedures”). With a frequency of 10<sup>−7</sup>, mutant colonies appeared that apparently converted adenine efficiently into guanine nucleotides despite the blocked deoD pathway and the presence of histidine in the medium. Sixty of such independent mutants were characterized further and found to belong to three distinct classes as described in the following.

Class I: Mutants with a Deregulated Histidine Biosynthetic Pathway—Half of the isolated mutants lost the ability to grow with adenine as a purine source upon introduction of the hisG::Tn10 allele from NK5526 (Table I), and for several of these mutants the responsible mutation was mapped to the hisG gene itself (data not shown). Thus, the feedback regulation of the HisG enzyme was probably eliminated in this class of mutants to allow an increased flux through the histidine biosynthetic pathway even in the presence of histidine. These mutants were not characterized further.

Class II: Mutants with Increased Adenine Deaminase Activity Caused by Mutations Closely Linked to the yicP Gene—The remaining mutants did not lose the ability to grow on adenine upon introduction of a hisG::Tn10 or a deoD::cam mutation (data not shown), suggesting that a novel third pathway for conversion of adenine into guanine nucleotides had been activated. Based on differences with respect to growth and colony morphology, these mutants could be divided into two distinct classes (II and III) consisting of 20 and 10 independent clones, respectively. The Class II mutants, like the parent strain, grew well on a variety of carbon sources and formed normal non-mucoid colonies. Genetic analysis revealed that the responsible mutation in the Class II mutants showed a high cotransduction frequency with the ilvB locus (data not shown), which is compatible with a location in the immediate vicinity of the yicP gene. Thus, we hypothesized that the novel activated pathway in Class II mutants might involve direct deamination of adenine to hypoxanthine by the yicP gene product.

In support of this notion, we found that a crude extract of one of the Class II mutants, CN2388-2, efficiently converted adenine into hypoxanthine with no apparent formation of any intermediate products, corresponding to a 20-fold increase of the cellular adenine deaminase activity compared with the parent strain, CN1980 (Fig. 2, left and center panels). Furthermore, we subjected a derivative of CN2388-2 to transposon mutagenesis with mini-Tn10 cam and isolated a clone that had specifically lost the ability to grow with adenine as a purine source while retaining the ability to utilize hypoxanthine. Subsequent analysis revealed that the cam insert in this strain, CN2451, had indeed disrupted the yicP gene (see “Experimental Procedures”). Back transduction of the yicP::cam allele into all the Class II mutants eliminated their ability to use adenine as a purine source (data not shown), confirming that the original phenotype was caused by activation of the yicP gene, which we propose be renamed ade.

This conclusion was further corroborated by cloning of the chromosomal ade<sup>e</sup> gene and its native promoter in a multicopy plasmid, pAde (see “Experimental Procedures”). Introduction of this plasmid increased the adenine deaminase activity of CN1980—30-fold from 0.5 to 15.4 µmol/min/g of dry weight and enabled the transformant to grow well in glucose minimal medium with adenine as the sole source of purines at a rate comparable with that of a purine-prototrophic strain (data not shown). These results indicated that the wild type ade<sup>e</sup> gene encodes a functional adenine deaminase but is simply too poorly expressed at normal gene dosage to satisfy the cellular purine requirement, which is on the order of 4 µmol/min/g of dry weight (33). Indeed, the low but significant adenine deaminase activity in the wild type strain, CN1980, was completely abolished by the ade::cam disruption in CN2481 (compare Figs. 2, left and right panels). In agreement with the results of Matsui et al. (22), the E. coli adenine deaminase was strongly dependent on manganese ions for activity (see “Experimental Procedures”), as also observed for the homologous enzyme from B. subtilis (36).
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was independent of the orientation of the insertion element. The cellular adenine deaminase activity of a representative Class III mutant, CN2388-16 (Table I), was found to be 3.8 times higher than that of wild type, indicating that the responsible activating mutation was unlinked to the ade promoter region (Fig. 3). In 12 of these mutants the ade gene was activated by integration of an IS1 element within the first 100 base pairs surrounding the divergent neighboring gene, yicO. Two mutants were activated by insertion of IS4 or IS5 in the same region of yicO, whereas one mutant contained an IS5 insertion in the intercistronic region between ade and yicO. The latter insertion was the only one found to be located downstream of the ade promoter. In addition we found two Class II mutants with unidentified insertions or rearrangements in yicO that could not be spanned by PCR amplification as well as three mutants that apparently contained an amplification of the entire ade region including the ilvB locus (data not shown).

The finding that all the identified Class II mutations mapped outside of the structural ade gene underscored the cryptic nature of this locus was caused by a low level of expression rather than by malfunctioning of the encoded gene product. It is also noteworthy that activation could occur by insertion of IS elements within an extended region of 160 base pairs surrounding the ade promoter and that this activation, at least for IS1, was independent of the orientation of the insertion element.

Class III; Mutants with Increased Expression of the ade Gene Caused by Mutations in the Unlinked hns Gene—The Class III mutants were clearly distinguishable from the parent strain and the other mutant classes by their formation of mucoid colonies on LB plates and their poor growth with glycerol or succinate as carbon sources irrespective of the purine source.

Accordingly, we mapped the mutation responsible for the increased ade deaminase activity and the other phenotypes of CN2388-16 to the 27-min region of the genome immediately clockwise of the supF marker (data not shown). This corresponds to the location of the hns gene, mutations in which are known to cause increased formation of capsular polysaccharides and poor growth on gluconeogenic carbon sources (37). Thus, we inferred that the Class III mutants might contain loss-of-function mutations in the hns gene, and this was confirmed by PCR amplification of the hns gene in CN2388-16 and another Class III mutant, CN2388-11, using the primers hns-RV + hns-H3 (Table II). DNA sequencing of the PCR fragments revealed that the hns gene in both cases had been disrupted by insertion of an IS1 element in the region encoding the C-terminal DNA binding domain of H-NS, leaving a truncated reading frame of 126 or 108 codons, respectively (plus additional codons encoded by the IS1 sequence).

In agreement with these results, we found that the cellular ade deaminase activity increased 11-fold upon introduction of the well characterized hns205::Tn10 allele into CN1980 (Table III). This hns disruption allele, which codes for a truncated H-NS protein of only 93 amino acid residues (38), also mimicked the other phenotypes of the selected Class III mutants with respect to increased mucoidicity and poor growth on glycerol or succinate. All these results indicated that the cryptic nature of the ade gene in wild type strains is due to H-NS-mediated gene silencing.

**IS Insertions Antagonize H-NS-mediated Repression of the ade Gene**—To investigate if the IS insertions of the Class II mutants activated the ade gene independently of the H-NS-mediated regulation, the hns205::Tn10 allele was transduced into three of the Class II mutants, CN2388-2 and CN2388-15, which are activated by an IS1 insertion, and CN2388-6, which is activated by IS4. In the hns background, the insertions in these strains gave rise to a dramatic increase of the cellular ade deaminase activity, ranging from 10- to 27-fold (Table III). However, the enzymatic activity produced from these alleles only increased by an additional 1.4–2.4-fold upon introduction of the hns205::Tn10 allele, which should be contrasted with the more than 10-fold stimulation seen for the wild type ade gene upon disruption of hns in CN1980 (Table III). Thus,
from the chromosomal gene in the very different CN1980 background (Table III). Thus, the region cloned on pAd, extending from the ade-R1 primer region (Fig. 3) to immediately downstream of the ade reading frame is sufficient to confer H-NS-mediated repression.

The target for the H-NS-mediated regulation was further defined by construction and characterization of ade-lacZ gene fusions (Fig. 4). The data for pCN2421 showed that the 231-base pair-long region bounded by the ade-R1 and the ade-H3 primer targets (Fig. 3) was sufficient to give more than 10-fold H-NS-mediated regulation of the ade-lacZ fusion. The inclusion of an additional 853 base pairs of yicO sequence upstream of the ade promoter in pCN2534 did not significantly affect the regulation by H-NS but reduced gene expression 2-fold both in the wild type and hns mutant background. These results corroborated the finding that sequences upstream of the ade-R1 primer region were not specifically required for H-NS-mediated repression and further established that sequences downstream of codon 22 in the ade gene were also dispensable for this regulation.

In agreement with these results, we found that the yicO-2::IS1 insertion stimulated expression of the ade-lacZ fusion in pCN2422 to the same extent as it stimulated expression of the chromosomal ade gene in CN2388–2 (compare Fig. 4 and Table III). The presence of the IS element almost eliminated the H-NS-mediated regulation of the lacZ fusion to a mere 1.4-fold and apparently increased the intrinsic strength of the ade promoter as seen from the 3-fold higher expression of pCN2422 compared with pCN2421 in the hns mutant background. The virtual abolition of H-NS-mediated repression of pCN2422 was not an artifact caused by the very high expression level of this construct. A low copy derivative of this plasmid, pCN2515, was similarly unresponsive to the hns mutation (Fig. 4). It might be imagined that the activating effect of the IS insertions derived from disruption of the divergent yicO gene if the YicO protein was somehow required for silencing of the ade gene. However, pCN2422 contains only the first 23 codons of yicO, and yet, the yicO::IS1 disruption in this plasmid had a similar activating effect as in the chromosomal context. This result strongly suggests that the IS insertions activated the neighboring ade promoter by a cis-effect independently of the YicO protein.

Finally, it should be noted that the stimulating effect of the hns mutation on expression of the ade-lacZ fusions was not caused by an unspecified effect on plasmid copy numbers or on
β-galactosidase synthesis as such. Expression of the lacZ gene both in the medium-copy vector, pCN302, and in the low-copy vector, pC2423, was actually reduced by ~30% in the hns background (data not shown).

Effect of an IS Insertion and the H-NS Protein on Transcription from the ade Promoter—To investigate how the IS sequences and the H-NS protein modulate ade gene expression, we mapped by primer-extension analysis the 5'-ends of ade-lacZ fusion transcripts produced in a wild type or a hns mutant background (Fig. 5). In the wild type background the unactivated fusions pCN2421 and pCN2534 only gave rise to a very faint signal that was hardly visible on the reproduction in Fig. 5 (but clearly visible on longer exposures). However, the same signal increased greatly in strength in the hns mutant background (Fig. 5). In the wild type background the unactivated fusion pCN2515 showed a strong signal at 120 nucleotides upstream of the ade initiation codon, which is even more visible on the reproduction in Fig. 5, whereas pC2534 contains an additional 853 base pairs of upstream yicO sequence.

These results indicate that the absence of the H-NS protein or the presence of the activating IS1 insertion caused a marked derepression of the ordinary ade promoter rather than promoting transcription from alternative start sites. In particular there was no indication that transcription originated from within the activating IS element in pCN2515 (pCN2422). In this connection it is intriguing that some of the IS1 insertions were located 83–120 nucleotides upstream of the −35 signal (Fig. 3), which is even upstream of the region that was sufficient for H-NS-mediated silencing of the ade gene (Fig. 4). These findings suggest that the IS1 insertions somehow modulated the DNA structure in the downstream ade promoter region to stimulate transcription and interfere with H-NS-mediated silencing.

**DISCUSSION**

Physiological Role of the E. coli ade Gene—Although the ade gene is cryptic in E. coli, the present work demonstrates that the encoded protein is a fully functional manganese-dependent
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protein binding is involved in IS1-mediated activation, it seems more likely that the crucial protein is the IS1-encoded transposase, in analogy with IS5-mediated activation.

Interestingly, the IS1 and IS4 elements tested here also seemed to stimulate transcription from the ade promoter independently of the H-NS protein (Table III). Conversely, the upstream yicO region between the ade-R12 and ade-R1 target sites negatively affected ade-lacZ expression independently of H-NS (Fig. 4). These findings suggest that the very AT-rich ade promoter region was particularly sensitive to changes in DNA topology imposed by neighboring DNA sequences, perhaps because it was particularly prone to unwinding or bending.

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