

## Translation of the *adhE* Transcript To Produce Ethanol Dehydrogenase Requires RNase III Cleavage in *Escherichia coli*

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**Previous studies have shown that the *adhE* gene, which encodes a multifunctional protein with ethanol dehydrogenase activity, is under transcriptional regulation. The level of dehydrogenase activity in cells grown fermentatively is about 10-fold higher than that in cells grown aerobically. In these studies, we mapped the promoter to a region well upstream of the protein-coding region of *adhE*. Unexpectedly, in mutants lacking the endoribonuclease RNase III, no significant ethanol dehydrogenase activity was detected in cells grown anaerobically on rich (Luria-Bertani) medium supplemented with glucose, even though *adhE* mRNA levels were high. Indeed, like  $\Delta$ *adhE* mutants, strains lacking RNase III failed to grow fermentatively on glucose but grew on the more oxidized carbon source glucuronate. Computer-generated secondary structures of the putative 5' untranslated region of *adhE* mRNA suggest that the ribosome binding site is occluded by intramolecular base pairing. It seems likely that cleavage of this secondary structure by RNase III is necessary for efficient translation initiation.**

During anaerobic growth in the absence of exogenous electron acceptors, the facultative anaerobe *Escherichia coli* produces fermentation products such as ethanol, glycerol, formate, acetate, D-lactate, succinate, CO<sub>2</sub>, and H<sub>2</sub> (4, 39). Ethanol is derived from acetyl coenzyme A by two consecutive NADH-dependent reductions catalyzed by the Fe<sup>2+</sup>-containing ethanol dehydrogenase protein comprising 891 amino acids (7, 10, 16, 21, 31, 34). Surprisingly, this protein also functions as a deactivase of pyruvate-formate lyase (16, 17). The *adhE* gene, encoding the multifunctional protein, is located at 27.9 min on the chromosome and has been sequenced previously (10, 17, 20). During fermentative growth on sugars, ethanol dehydrogenase is abundantly synthesized (reaching about 3 × 10<sup>4</sup> copies per cell) and assembles into large helical rods called spiroosomes, which are about 0.015 μm in width and up to 0.22 μm in length (16, 18, 22).

Studies with *lac* fusions have shown that regulation of *adhE* expression occurs at the transcriptional level and that anaerobic expression is about 10-fold higher than aerobic expression. Moreover, aerobic transcriptional repression (or anaerobic induction) is not caused by the presence of O<sub>2</sub>, since anaerobic nitrate respiration also strongly curtails transcription (3, 20). Anaerobic stimulation of *adhE* expression therefore seems to be related to the degree of accumulation of reducing equivalents. The results of two independent studies, one with *Klebsiella pneumoniae* (23) and one with *E. coli* (20), suggested that the NADH concentration or the NADH/NAD ratio control the redox signal. A crucial test of this hypothesis requires the identification of the *trans*-regulatory element for *adhE* and in vitro demonstration of an allosteric effect of the nicotinamide nucleotide on the putative transcriptional regulator. In addition to transcriptional regulation, the half-life of ethanol dehydrogenase is probably also shortened by oxidative inactiva-

tion, as observed for the corresponding protein of *K. pneumoniae* (13). To explore further the transcriptional regulation of *adhE*, we decided to map the extent of the promoter region and characterize the transcriptional start site. During the course of this study, we discovered that RNase III plays a critical role in *adhE* gene expression.

**Promoter mapping.** A stretch of 904 nucleotide pairs (base pairs) upstream of the *adhE* coding region has previously been shown to contain all necessary information for anaerobic induction of *adhE* transcription (3). To delineate the promoter region more precisely, we analyzed the expression of various *adhE'*-*lacZ* transcriptional fusions, containing different lengths of the upstream region, each inserted in a λ prophage integrated at the *att* λ site (Fig. 1; Table 1). In the resulting lysogens, anaerobic induction of φ(*adhE'*-*lacZ*) occurred when the coding region was preceded by 375 bp of the upstream sequence of *adhE*, but little induction or expression occurred when only 182 bp of the upstream region remained (Fig. 2). Thus, the *adhE* promoter appears to be confined to within a 375-bp stretch preceding the open reading frame of *adhE*.

**Analysis of transcriptional start site.** Primer extension experiments were performed to map the 5' end(s) of *adhE* mRNA. Preliminary experiments involved the use of total RNA isolated from anaerobically grown cells of strain MC4100 and primers spanning positions +2 to +21, +18 to +47, or +56 to +72 (position 0 corresponding to the first nucleotide A of the ATG start codon). These analyses yielded multiple bands by electrophoretic separation of the primer extension products. With all three primers, the longest transcript detected began with a G nucleotide at position -188. Shorter mRNAs, which might represent degradation products of the long transcript, were also observed. As expected from the previous report on φ(*adhE'*-*lac*) transcription (3), all of the transcript signals were barely detectable when the cells were grown aerobically or anaerobically with nitrate as the terminal electron acceptor (data not shown).

Because no promoter consensus could be discerned in the vicinity of position -188, we considered the possibility that even the longest primer extension product might correspond not to an mRNA 5' end but to the site of a structural imped-

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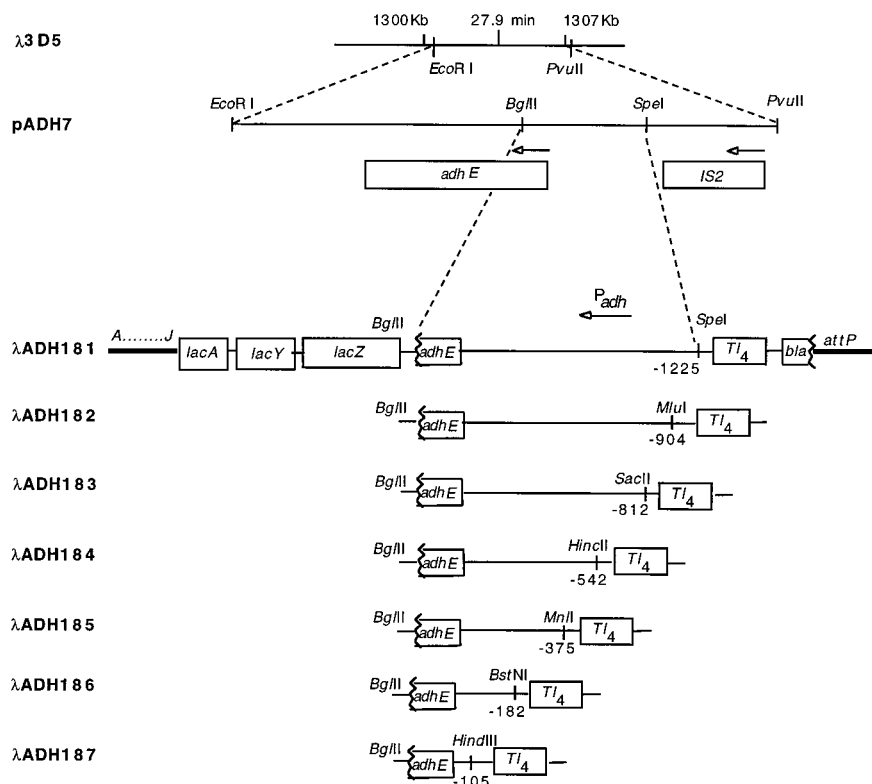


FIG. 1. Construction of *adhE'*-*lacZ* fusions with different lengths of the promoter and 5' untranslated regions. An 8-kb *EcoRI*-*PvuII* insert containing the entire *adhE* gene was excised from  $\lambda$ 3D5 DNA (19) and ligated between the *EcoRI* and *EcoRV* sites of the plasmid vector pBluescript SK(+) to yield pADH7 (3). The *adhE* insert of pADH7 was isolated and cleaved at the same *Bgl*II (*Bg*) site on the 3' side but at different restriction sites on the 5' side. Each fragment, containing a 5' segment of the *adhE* coding region along with different lengths of upstream sequence, was ligated between the *Sma*I (*Sa*) and *Bam*HI sites of the plasmid vector pRS415. Thus, the resulting product bears, in the 5' to -3' direction, a partial *bla* sequence, the transcriptional terminator *Tl*<sub>4</sub>, a partial *adhE* sequence, and a promoterless *lacZYA* operon (35). Each of the resulting plasmids was introduced into strain MC4100 by electroporation, and the transformants were infected with  $\lambda$ RS45, which contains *bla'* and *lac* sequences. In vivo homologous recombination between  $\lambda$ RS45 and deletant plasmids (pADH181 to pADH187) resulted in a prophage ( $\lambda$ ADH181- $\lambda$ ADH187) bearing the *adhE'*-*lac* operon fusion integrated at the *att* site. The  $\lambda$ ADH181 lysogen has an insert of *adhE* starting at the *Ssp*I site, 1,225 bp upstream of the coding region of *adhE* and extending to the *Bgl*II site at nucleotide position +493 within the *adhE* coding region. The insert in other integrated prophages bears a similar fusion beginning at position -904 (cut at *Mlu*I) position -812 (cut at *Sac*II), position -542 (cut at *Hinc*II), position -375 (cut at *Mnl*I), position -182 (cut at *Bst*NI), or position -105 (cut at *Hind*III).

iment to primer extension by reverse transcriptase. We thus decided to investigate whether the true transcription start site(s) might actually be located further upstream. To test this possibility, additional primer extension experiments were carried out with two primers positioned at a greater distance upstream (spanning positions -199 to -182 or -141 to -117) of the coding region. Figure 3 shows that whereas the downstream primer revealed two transcripts, one starting at an A nucleotide at position -292 (arrows labeled 2 and 3) and the other at a G nucleotide at position -188 (arrows labeled 1 and 4), the upstream primer revealed only one transcript starting at an A nucleotide at position -292. A putative -10 promoter sequence (TACAAT) was found at the appropriate distance upstream of position -292, although a sequence resembling the -35 box was not evident. The absence of the latter, however, would not be unusual if the promoter was under the control of a transcriptional activator protein(s) (29). Further analysis will be required to determine whether position -292 in fact corresponds to the site of transcription initiation. In any event, the results from the primer extension experiments are consistent with the data from the 5' deletion experiments, which indicate that the *adhE* promoter region extends beyond position -182 but not -375.

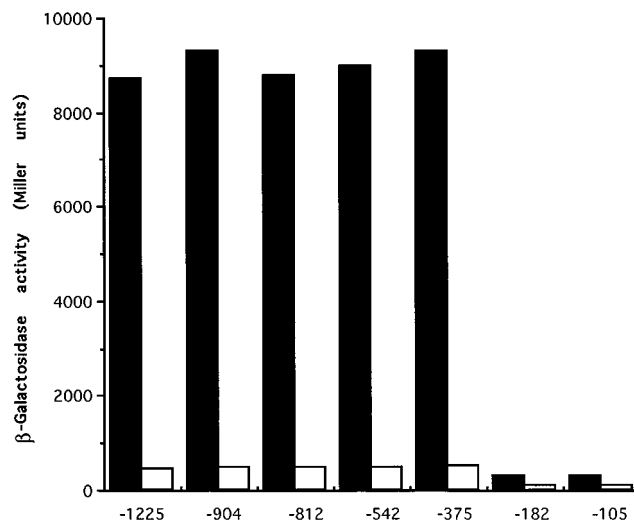


FIG. 2. Anaerobic induction of *adhE'*-*lacZ* fusions bearing different lengths of the promoter and 5' untranslated regions.  $\beta$ -Galactosidase activity was assayed (26) in each of the lysogens (ECL2030A to ECL2036A) grown anaerobically or aerobically at 37°C in LB-0.1 M MOPS (pH 7.4)-0.4% glucose (LB-glucose medium). The numbers on the abscissa represent the number of *adhE* base pairs present upstream of the *adhE* coding region. Closed bar, anaerobic growth; open bar, aerobic growth.

TABLE 1. *E. coli* K-12 strains, phages, and plasmids

Strain, phage, or plasmid	Relevant genotype	Source or reference
<b>Strains</b>		
CAG18570	MG1655 <i>zff-3139::Tn10 kan</i>	36
CH1827	<i>araD139 Δ(araABC-leu) 7697 Δ(lac)X74 galU galK hsdR rpsL150 thi zce-726::Tn10</i>	28
CH1828	Same as CH1827 but <i>ams-1</i> [i.e., <i>rne(Ts)</i> ]	28
MC4100	F <sup>-</sup> <i>araD139 Δlac U169 rpsL150 relA1 ffb-5301 deoC1 ptsf25</i>	M. J. Casadaban
RS6184	<i>Δ(lac-pro)ara thi glyA::Tn5</i>	R. W. Simons
RS6181	Same as RS6184 but <i>rnc-105</i>	R. W. Simons
RS6521	Same as RS6184 but <i>rnc::Tn10</i>	R. W. Simons
ECL2006	Same as MC4100 but <i>rnc::Tn10</i>	P1 (RS6521) × MC4100
ECL2007	Suppressor of anaerobic growth defect of ECL2006 (mutation uncharacterized)	This study
ECL2020	Same as ECL2007 but <i>rnc<sup>+</sup> zff-3139::Tn10 kan</i>	P1(CAG18570) × ECL2007
ECL2022	Same as ECL2007 but <i>rnc::Tn10 zff-3139::Tn10 kan</i>	P1(CAG18570) × ECL2007
ECL2030A	Same as MC4100 but <i>λADH181</i>	This study
ECL2031A	Same as MC4100 but <i>λADH182</i>	This study
ECL2032A	Same as MC4100 but <i>λADH183</i>	This study
ECL2033A	Same as MC4100 but <i>λADH184</i>	This study
ECL2034A	Same as MC4100 but <i>λADH185</i>	This study
ECL2035A	Same as MC4100 but <i>λADH186</i>	This study
ECL2036A	Same as MC4100 but <i>λADH187</i>	This study
<b>Phage</b>		
λRS45	<i>lacZ' lacYA<sup>+</sup> bla'</i>	35
λADH181	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details see Fig. 1)	This study
λADH182	Same as λADH181	This study
λADH183	Same as λADH181	This study
λADH184	Same as λADH181	This study
λADH185	Same as λADH181	This study
λADH186	Same as λADH181	This study
λADH187	Same as λADH181	This study
<b>Plasmids</b>		
pRS415	Ap <sup>r</sup> <i>lac ZYA<sup>+</sup></i>	35
pAM20	<i>adhE'-cat</i> operon fusion [pBluescript II KS (-)]	This study
pAM23	<i>adhE'::cat</i> protein fusion [pBluescript II KS (-)]	This study
pAM24	<i>adhE'::cat</i> protein fusion (pRS415 derivative)	This study
pADH108	Ap <sup>r</sup> <i>adhE'-lacZ</i>	Y.-M. Chen
pADH181	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH182	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH183	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH184	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH185	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH186	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study
pADH187	Ap <sup>r</sup> <i>adhE'-lacZ</i> (for details, see Fig. 1)	This study

**Effects of endoribonucleases on *adhE* mRNA.** Since some or all of the short transcripts observed in the primer extension experiments might have resulted from in vivo RNA processing, we examined *adhE* mRNA in cells deficient in RNase III (*rnc* product) or RNase E [*rne(Ts)* product], two endoribonucleases implicated in the degradation of many different bacterial and phage mRNAs and in the processing of rRNAs (1, 5, 24, 28, 30, 38; for a review, see reference 6). An *rnc::Tn10* (null) allele was transduced from strain RS6521 to the standard *rnc<sup>+</sup>* strain (MC4100) used in this study to give strain ECL2006. During the course of the work, we noticed that both strain RS6521 and the transductant ECL2006 were severely impaired for anaerobic growth in Luria-Bertani (LB) or LB-glucose (LB with 0.4% glucose in 0.1 M MOPS [morpholinepropanesulfonic acid] at pH 7.5) medium. To circumvent this anaerobic growth defect associated with the *rnc* mutation, we selected for a spontaneous mutant of ECL2006 with improved anaerobic growth in LB-glucose medium. This suppressor mutant (strain ECL2007) remained tetracycline resistant, suggesting retention of the *rnc::Tn10* allele.

In order to analyze the effect of RNase III on *adhE* mRNA

in the same suppressor background, a P1 lysate of strain CAG18570 bearing an *rnc<sup>+</sup>* allele linked to *zff-3139::Tn10(kan)* was used to transduce strain ECL2007 (*rnc::Tn10*). Two kinds of kanamycin-resistant transductants were obtained: one (strain ECL2022) was resistant to tetracycline and therefore should have the genotype *rnc<sup>+</sup> zff-3139::Tn10(kan)*, and the other (strain ECL2020) was sensitive to tetracycline and therefore should have the genotype *rnc::Tn10 zff-3139::Tn10(kan)*. These isogenic *rnc<sup>+</sup>* and *rnc::Tn10* strains bearing the undefined suppressor mutation(s) were grown anaerobically in LB-glucose medium for preparation of RNA extracts. When 5' extension experiments were carried out with a complementary primer spanning positions +56 to +72, most of the shorter mRNAs observed in the *rnc<sup>+</sup>* strain were not seen in the isogenic *rnc::Tn10* strain (Fig. 4).

Similar results were obtained when the *rnc<sup>+</sup>* strain RS6184 was compared with an isogenic mutant (strain RS6181) bearing an *rnc-105* point mutation that inactivates RNase III. Control experiments showed that the *rnc::Tn10* insertion mutation did not affect anaerobic induction of the *adhE* mRNAs and that

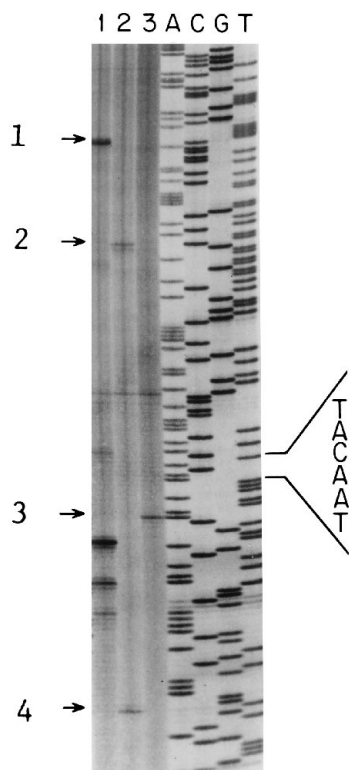


FIG. 3. Mapping of the 5' ends of *adhE* mRNA. Primer extensions were performed with SuperScript II RNase H<sup>-</sup> reverse transcriptase (Gibco BRL), with total mRNA from MC4100 and oligonucleotide primers spanning different regions of *adhE*. Extension products were labeled with <sup>35</sup>S (25). Lanes: 1, with a primer (5'-TGGCATATTCACGCTGG-3') complementary to positions +56 to +72 (with 0 corresponding to nucleotide A of the start codon ATG), several bands were observed; 2, with a primer (5'-ACACTCACTGTGATTTACTA AAAGA-3') corresponding to positions -141 to -117, two bands were observed; 3, with a primer (5'-AGGTGGCGTAAGCAAGAT-3'), corresponding to positions -199 to -182, only a single band was observed. Arrows numbered 1 and 4 indicate the positions of *adhE* mRNAs that begin at position -188, and arrows numbered 2 and 3 indicate the positions of *adhE* mRNAs that begin at position -292. The base sequence on the right, showing the putative *adhE* promoter, was generated with the third primer (5'-AGGTGGCGTAAGCAAGAT-3'). The band appearing between arrows 2 and 3 is probably artifactual, since its position on the gel is independent of the primer used.

these mRNAs were only barely detectable when the cells were grown anaerobically in the presence of nitrate (data not shown). The nitrate effect would be predicted from a previous study on nitrate repression of  $\phi(adhE'-lacZ)$  expression (3).

When a strain producing a temperature-sensitive variant of RNase E was tested at the nonpermissive temperature of 42°C, no change in the *adhE* mRNA banding pattern was observed (Fig. 4).

**Mechanism of RNase III control of *adhE* expression and its possible physiological significance.** In view of the fact that RNase III cleaves double-stranded RNA (30), the simplified pattern of *adhE* mRNAs in extracts from *rnc* cells suggests that the *adhE* transcript might contain a secondary structure element that is a substrate of this nuclease. In principle, RNase III activity could affect gene expression in one of two ways. First, it could decrease the mRNA half-life and thereby lower the steady-state level of the protein product. For instance, it is known that RNase III regulates expression of the  $\lambda$  *int* gene at the level of mRNA stability and autoregulates RNase III synthesis by cleaving its own mRNA, thereby triggering its degradation (2, 32). Alternatively, cleavage of the secondary struc-

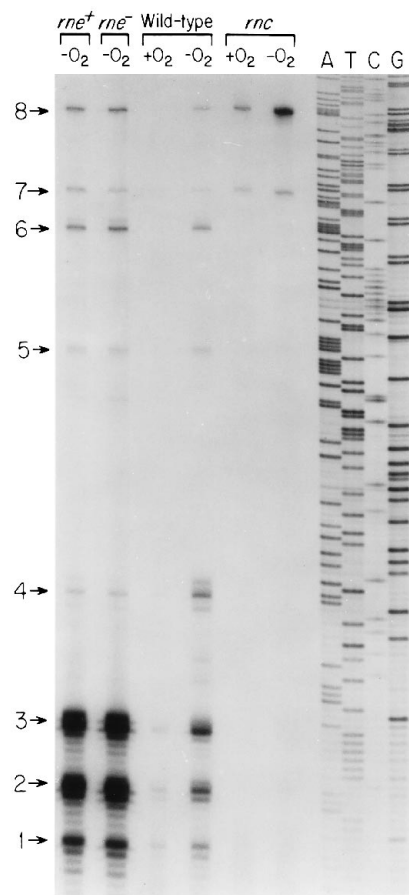


FIG. 4. Band patterns of primer extension experiments performed with RNA extracts from *mc*<sup>+</sup> *me*<sup>+</sup> strains and mutants lacking RNase E or RNase III activity. Primer extensions were carried out under conditions similar to those described in the legend to Fig. 3, except that a primer (5'-TACACGCTCTAC GAGTGGCGTTAAGTTCAGC-3') complementary to positions +18 to +47 was used. Lanes labeled *me*<sup>+</sup> and *me*<sup>-</sup>, strains CH1827 (*me*<sup>+</sup>) and CH1828 [*me*(Ts)], respectively, were grown anaerobically at 30°C and shifted to 42°C at mid-exponential phase. The cells were harvested 1 h after the temperature shift. For lanes labeled wild-type, strain MC4100 (*mc*<sup>+</sup>) was grown under aerobic (+O<sub>2</sub>) or anaerobic (-O<sub>2</sub>) conditions at 37°C; lanes labeled *rnc*, the *mc::Tn10* mutant cells were grown under aerobic (+O<sub>2</sub>) or anaerobic (-O<sub>2</sub>) conditions at 37°C. Major primer extension products are marked by arrows (1 to 8). Band 8, the longest transcript detected in these experiments, corresponds to *adhE* mRNA beginning at position -188.

ture in the transcript might expose or occlude a signal for translation initiation. For example, cleavage could liberate a ribosome binding site (RBS) concealed by base pairing. Among the mRNAs of *E. coli* and its phages, there is ample precedent for inhibition of translation initiation by secondary structure formation (8, 9, 12, 14, 27, 32, 37).

The MFold program (40) was used to model possible secondary structures of the 5' segment of the primary *adhE* transcript. Among the five thermodynamically most favorable steric configurations predicted for the nontranslated region, all shared one common feature: the RBS is sequestered by intramolecular base pairing with the same segment of the 5' untranslated region (Fig. 5). The formation of these structures would be expected to inhibit translation. If so, cleavage by RNase III might liberate the RBS and allow it to bind 30S ribosomal subunits.

**Effects of *rnc* mutation on AdhE protein synthesis and fermentative growth on single carbon and energy sources.** To test

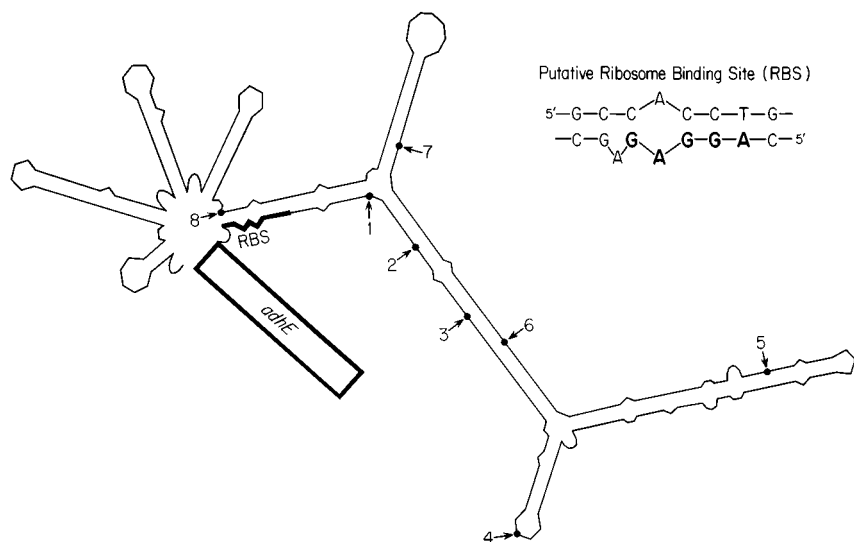


FIG. 5. Possible secondary structure of the 5'-untranslated region of the primary *adhE* transcript. The thermodynamically most favorable mRNA secondary structure predicted by the Mfold program (40) is shown. The primary sequence includes 292 nucleotides upstream of the coding region. The five most favorable structures share a common feature: the RBS is sequestered in identical stem structures, which should impede translation initiation. The numbers 1 to 8 correspond to the ends of the major primer extension products shown in Fig. 4.

whether an RNase III deficiency would actually impede the translation of the *adhE* transcript, we assayed the level of ethanol dehydrogenase activity in extracts prepared from strains ECL2020 (*mc*<sup>+</sup>) and ECL2022 (*mc*::*Tn10*) grown anaerobically in LB-glucose medium. The *mc*<sup>+</sup> strain, ECL2020, showed an activity level of 11  $\mu\text{mol}/\text{min}/\text{mg}$  of protein, which was close to that of the reference wild-type strain MC4100 (10  $\mu\text{mol}/\text{min}/\text{mg}$  of protein). In contrast, the *mc* mutant, ECL2022, exhibited no significant ethanol dehydrogenase activity, even though high levels of *adhE* mRNA are present in these cells.

Since the reduction of each molecule of acetyl coenzyme A to ethanol oxidizes two molecules of NADH, ethanol dehydrogenase is expected to be most important during fermentation of carbon and energy sources that contain a large number of reducing equivalents. For instance, it has previously been demonstrated that whereas the fermentation of mannitol (generating three reducing equivalents per mole of pyruvate formed) and glucose (generating two reducing equivalents per mole of pyruvate formed) requires the intervention of AdhE, the fermentation of glucuronate (generating no reducing equivalents per mole of pyruvate formed) does not (7, 11). We found that the *mc*::*Tn10* mutant, like a  $\Delta$ *adhE* or an *adhE*::*kan* mutant, failed to grow anaerobically on mannitol or glucose as a sole carbon and energy source but did grow on glucuronate under the same conditions. All three strains, however, grew at similar rates aerobically (data not shown). Thus, these growth experiments also point to a lack of ethanol dehydrogenase activity in *mc* cells. Together with the results of our 5'-end mapping experiments, the failure of *mc* cells to produce ethanol dehydrogenase despite the presence of high levels of *adhE* mRNA supports the RBS liberation hypothesis.

To test this hypothesis further, we exploited a plasmid-borne *adhE'*-*cat*-*lacZ* triple fusion in which *adhE'* and the *cat* coding sequences were placed in frame. Synthesis of the AdhE':CAT hybrid protein was initiated from the *adhE* RBS, whereas the synthesis of chloramphenicol acetyltransferase (CAT) was initiated from the *cat* RBS. On the other hand, the synthesis of  $\beta$ -galactosidase was initiated only at the *lacZ* RBS. Enzyme

assays of  $\beta$ -galactosidase activity showed that transcription of the triple fusion construct responded correctly to anaerobic induction and that the level of  $\beta$ -galactosidase activity was independent of the *mc* allele. Western blot (immunoblot) analysis with anti-CAT antibodies showed likewise that the CAT protein was synthesized in both *mc*<sup>+</sup> and *mc* strains but that

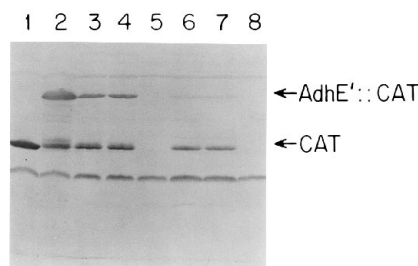


FIG. 6. Effect of an *mc* null mutation on the translation of an *adhE'*-*cat*-*lacZYA* fusion. The plasmid pADH108 (a pRS415 derivative), bearing an *adhE'*-*cat*-*lacZYA* operon fusion (2a), was used to construct an *adhE'*-*cat* protein fusion by insertion of the oligonucleotide 5'-CCAGCTGG-3' (New England Biolabs) into the *EcoRV* site located in the *adhE* coding region between the *MuI* and *BglII* sites. In the resulting plasmid, pAM24, the *adhE'* and *cat* sequences were placed in frame. Consequently, translation of the *adhE'*-*cat*-*lacZYA* mRNA can be initiated at the *adhE* RBS to yield a long AdhE'-CAT hybrid polypeptide or at the *cat* RBS to yield the wild-type CAT protein. (The translation of *lacZ* is initiated at its own RBS.) Plasmid pAM24 was then used to transform the isogenic strains ECL2020 [*mc*<sup>+</sup> *zff-3139*::*Tn10(kan)*] and ECL2022 [*mc*::*Tn10 zff-3139*::*Tn10(kan)*]. Strains were grown anaerobically in LB-glucose medium to stationary phase to allow high-level transcription from P<sub>adhE</sub>. The AdhE'-CAT and CAT levels were monitored by Western blot analysis with antibodies prepared against CAT (5 prime  $\rightarrow$  3 prime, Inc). Lanes: 1, control with CAT alone expressed by ECL2020 (*mc*<sup>+</sup>)/pAM20 (The plasmid pAM20 is a pBlueScript II KS(-) derivative containing four tandem T<sub>14</sub> terminators of the *rmB* operon followed by a P<sub>adhE</sub>-*adhE'*-*cat* operon fusion.); 2, control with *adhE'*-*cat* and *cat* expressed by ECL2020 (*mc*<sup>+</sup>)/pAM23 (pAM23 is a pBlueScript II KS(-) derivative containing four tandem T<sub>14</sub> terminators of the *rmB* operon followed by a P<sub>adhE</sub>-*adhE'*-*cat* protein fusion.); 3 and 4, strain ECL2020 (*mc*<sup>+</sup>)/pAM24; 5, strain ECL2020 (*mc*<sup>+</sup>)/pRS415 (negative control); 6 and 7, strain ECL2022 (*mc*::*Tn10*)/pAM24; 8, ECL2022 (*mc*)/pRS415 (negative control). The most rapidly migrating band that appears in every lane is a cross-reacting protein in the host.

the AdhE'-CAT hybrid was abundantly synthesized only in *mc*<sup>+</sup> strains. These findings demonstrate that the *adhE* RBS is functional in cells containing RNase III but is inoperative in cells lacking this nuclease (Fig. 6).

A similar conclusion has recently been drawn for the control of expression of the *speF-potE* operon in *E. coli*. That operon encodes ornithine decarboxylase (known to be inducible by ornithine under anaerobic and acidic conditions) and polyamine transport protein. For *speF-potE*, however, overexpression of RNase III on a plasmid increased the translation of the operon by less than fourfold (15), in contrast to the essentially all-or-none effect that we have observed for the *adhE* operon.

The existence of an RNase III-dependent mechanism for controlling *adhE* translation encourages us to think that RNase III might play a regulatory role in modulating the expression of *adhE* and other genes in response to physiological signals other than oxygen. In this manner, RNase III might serve to integrate various response circuits. Future work should explore physiological conditions that may affect the level of cellular RNase III activity, with the objective of discovering yet another environmental parameter that regulates the level of the AdhE protein.

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