Volume 11 Number 1 1983 Nucleic Acids Research

Nucleotide sequence of the *ilvB* multivalent attenuator region of *Escherichia coli* K12

Craig A. Hauser and G. Wesley Hatfield

Department of Microbiology, College of Medicine, University of California, Irvine, CA 92717, USA

Received 14 September 1982; Revised and Accepted 1 December 1982

ABSTRACT

The ilvB gene of Escherichia coli K12 has been cloned into a multicopy plasmid. The regulation of the cloned gene by valine or leucine limitation and by catabolite repression is the same as for the chromosome encoded gene. The nucleotide sequence of a regulatory region preceding the ilvB structural gene has been determined. This DNA sequence includes a promoter, a region which codes for a putative 32 amino acid polypeptide containing multiple valine and leucine codons, and a transcription termination site. In vitro transcription of this region produces a 184 nucleotide terminated leader transcript. Mutually exclusive secondary structures of the leader transcript are predicted. On the basis of these data, a model for multivalent attenuation of the ilvB operon is presented. Data are presented which suggests that at least part of the postulated CRP-cyclic AMP binding site of the ilvB operon precedes the transcription start site by more than 71 base pairs.

INTRODUCTION

Three isozymes catalyze the first step common to the biosynthesis of both isoleucine and valine in Escherichia coli. These isozymes are: acetohydroxy acid synthase (AHAS) I, the ilvB gene product; AHAS II, the ilvG gene product; and AHAS III, the ilvHI gene products (1). Although all wild type Escherichia coli strains possess these three genes, E. coli K12 expresses only the AHAS I and AHAS III activities. These activities are both sensitive to feedback inhibition by valine, whereas the AHAS II isozyme (normally not expressed in E. coli K12) is resistant to inhibition by this end product (2).

Each of the AHAS genes is regulated by separate end product repression patterns. The expression of the ilvB gene (AHAS I) is repressed in the presence of valine and leucine. The ilvG gene (AHAS II) is repressed only in the presence of all three branched chain amino acids, isoleucine, valine, and leucine. The repression of the ilvHI genes (AHAS III) on the other hand requires only the presence of leucine (3). It has also been reported by Whitlow and Polglase (4) and by Freundlich (5), that the total AHAS activity expressed in E. coli K12 is subject to catabolite repression. Sutton and Freundlich have shown more recently that only the ilvB gene is subject to this control (6), and Newman et al. have shown that a cloned ilvB gene exhibits the same pattern of regulation (7). These are the only reports of a catabolite repressible amino acid biosynthetic gene.

© IRL Press Limited, Oxford, England.

0305-1048/83/1101-0127$2.00/0

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

To further elucidate the molecular mechanisms of ilvB gene regulation, we cloned a fragment of DNA containing this gene into a multicopy plasmid vector. We confirm that the cloned ilvB gene is regulated by multivalent repression and by catabolite repression. In addition, we present evidence that the multivalent end product repression of ilvB gene expression is mediated by an attenuator mechanism. This evidence includes DNA sequence and in vitro transcription analyses of the ilvB gene regulatory region.

MATERIALS AND METHODS

Materials

RNA polymerase was obtained from Miles laboratory and other enzymes were obtained from Bethesda Research Laboratories or New England Biolabs. Sequencing primer and dideoxynucleotides were obtained from Collaborative Research, Inc.

Bacterial Growth Conditions

The minimal media used was M63 described by Miller (8) supplemented with 5 μg/ml thiamine, 10 mM $MgSO_4$, and unless indicated, 0.5% glucose as the carbon source. All other carbon sources were used at 0.5%. All plasmid containing strains were grown in media containing either ampicillin 100 μg/ml, or kanamycin 30 μg/ml. Auxotrophic strains were supplemented with 50 μg/ml of appropriate amino acids. Amino acid limitation experiments were performed by harvesting mid log cultures by centrifugation, washing twice in prewarmed minimal media, and resuspending in media containing 50 μg/ml of the nonlimiting amino acids. Limiting isoleucine or leucine was 2.5 μg/ml, limiting valine was 5 μg/ml. Tryptophan limitation was effected by resuspension in media without tryptophan. The limited cultures were incubated at 37°C for 3 hr (approximately one doubling).

Enzyme Assays

The assay for acetohydroxy acid synthase was basically that of Stormer and Umbarger (9). Each 1.0 ml reaction mix contained 0.2 ml sonicated extract, 0.1 M potassium phosphate pH 7.5, 0.1 mM thiamine pyrophosphate, 20 μg flavin adenine dinucleotide, 40 mM sodium pyruvate, and 10 mM $MgCl_2$. After incubation at 37°C for 15 or 30 min the reaction was stopped by the addition of 0.2 ml of 50% H_2SO_4. This mixture was further incubated at 37°C for 30 min. The color development was performed using 1.6 ml H_2O, 1.0 ml 0.5% creatine hydrate, 0.6 ml 0.5% α-napthol in 10% NaOH, and .01-.1 ml of the stopped reaction mix. This mixture was placed at room temperature for 1 hr and the optical density at 540 nm was determined. A standard curve was generated using Eastman Kodak acetoin. Protein concentration was determined using the method of Bradford (10).

DNA Sequencing

DNA sequencing was performed according to the dideoxy chain termination method

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

of Sanger et al. (11). All regions of both strands of the DNA sequence shown in Figure 1 were sequenced from overlapping recombinant phage M13 clones. The RNA secondary structures shown in Figure 2 and 5 were confirmed by computer analyses using the Stanford Mol. Gen. Project and the NIH SUMEX-AIM Facility.

In Vitro Transcriptions

In vitro transcriptions were performed and analyzed on polyacrylamide urea gels as previously described (12) but using 30 μCi [α-^{32}P]CTP (400 Ci/mmole) and 10 μM cold CTP, along with 0.4 mM UTP, ATP, and GTP, or 0.8 mM ITP instead of GTP. Reactions were terminated after 10 min.

RESULTS

Cloning of the ilvB Gene

Since the *ilvB* gene has been reported to be closely linked to the *uhp* locus at 81.5 min on the *E. coli* chromosome (13) hybrid Col E1 plasmids containing randomly sheared *E. coli* DNA (14) which carry the *uhp* gene (J. Carbon, personal communication) were screened for the ability to complement the AHAS$^-$ *E. coli* K12 strain CU888 (15). An EcoRI endonuclease restriction fragment from the smallest complementing plasmid, pLC35-4, was subcloned into the plasmid pBR322. A Pst1 endonuclease restriction fragment from this plasmid was further subcloned into the plasmid pACYC177 (16). The resulting plasmid, pCH4, contains a 6.5 kb Pst1 insert encoding a protein with AHAS activity. To confirm that this plasmid encodes the *ilvB* gene product and not one of the other AHAS isozymes, the pH optima and valine sensitivity of the plasmid encoded AHAS activity in crude extracts of the AHAS deleted strain, CU888/pCH4, was determined. A pH optima of 7.3 and a 90% inhibition of enzyme activity by 1.5 mM valine (17), as well as the original chromosomal location of the cloned gene, confirm that the plasmid pCH4 indeed contains the *ilvB* gene.

Regulation of ilvB Expression on pCH4 by Amino Acid Limitation

In order to determine if the *ilvB* regulatory region on pCH4 is intact, the response of pCH4 encoded AHAS activity to individual branched chain amino acid limitations was examined. To perform these experiments this plasmid was transformed into the *ilv*GEDAYC deletion strain of T31-4-4 (18), T31-4-505. The phenotype of this strain (Ilv$^-$, Trp$^-$) allows not only limitation of the individual branched chain amino acids involved in the genetic regulation of this gene (valine and leucine) but also for nonregulating amino acids, tryptophan, and isoleucine. All the limitations were done in both strain T31-4-505 and the plasmid containing strain T31-4-505/pCH4 (Table 1) in order to show that the small amount of expression of *ilvB* and *ilvHI* from the chromosome does not significantly alter the results. Table 1 also shows that either valine or leucine limitation increases AHAS specific activity in crude extracts 6- and 4-fold, respectively,

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

Table 1. Regulation of AHAS Levels by Amino Acid Limitations

Growth Condition	Strain T31-4-505 AHAS sp act	Strain T31-4-505 Relative sp act[a]	T31-4-505/pCH4 AHAS sp act	T31-4-505/pCH4 Relative sp act[b]	T31-H-505 AHAS sp act	T31-H-505/pCH4 AHAS sp act
Excess ile, leu,val,trp	6.5	1.0	66.6	1.0	16.3	221.0
Limiting trp	1.4	0.2	23.1	0.3	-	-
Limiting ile	2.3	0.3	16.3	0.2	-	-
Limiting val	27.3	4.2	395.0	5.9	-	-
Limiting leu	21.3	3.3	268.0	5.0	-	-

[a] Relative to T31-4-505 grown with excess Ile, Val, Leu, Trp.
[b] Relative to T31-4-505/pCH4 grown with excess Ile, Val, Leu, Trp.
Sp. act. in nmoles acetoin/min/mg protein.

whereas isoleucine or tryptophan limitation actually decreases AHAS activity. This pattern of regulation, derepression by leucine or valine limitation but not by isoleucine or tryptophan limitation, is the same as data previously published for the regulation of the *ilvB* gene (19).

Effect of hisT on ilvB Expression

It has been shown that the *hisT* mutation prevents the modification of uridine to pseudouridine in certain tRNA isoacceptors in *E. coli* K12 (18). It has further been shown that derepression occurs in the presence of a *hisT* mutation for attenuated amino acid biosynthetic operons whose cognate tRNA normally contain pseudouridine (20,21). pCH4 was transformed into strain T31-H-505, an isogenic *hisT76* derivative of strain T31-4-505. Over a threefold derepression of *ilvB* in strain T31-H-505/pCH4 compared to T31-4-505/pCH4 was observed (Table 1). Therefore, these results are supportive evidence for the regulation of *ilvB* by an attenuator mechanism.

Catabolite Repression of ilvB on pCH4

The expression of the plasmid encoded *ilvB* gene in a Leu^- derivative of strain CU888/pCH4 was examined under different catabolite repressing conditions (Table 2). In the presence of excess leucine, there is a twofold increase in AHAS I specific activity after carbon source downshift from glucose to glycerol or succinate. The same decrease is observed by addition of 3 mM cyclic AMP to a glucose culture. Furthermore, a shift from glucose to the rich catabolite glucose-6-phosphate in the presence of excess

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

leucine, causes a decrease in AHAS I specific activity (Table 2). Further experiments demonstrated the requirement for both the cya and crp gene products (data not shown), as previously reported for the chromosome encoded ilvB gene (6). The approximately multiplicative effect of leucine limitation in the presence of different carbon sources suggest that the amino acid mediated derepression and the catabolite repression signals act at distinct regulatory sites.

Isolation of the ilvB Regulatory Region

Tn5 mutagenesis and subcloning of the plasmid pCH4 showed that the ilvB regulatory region is encoded within a 1.4 kb HincII-HindIII restriction fragment. The HincII site of this fragment was converted to a HindIII site with a synthetic HindIII linker and the entire fragment was cloned into the HindIII site of the galK transcription fusion vector pKO1 forming the plasmid pCH2 (22). This fragment is oriented in pCH2 such that fragment initiated transcription traverses the original HindIII site and regulates galK expression in a manner diagnostic of the ilvB regulatory region. This result defines the direction of ilvB transcription (HincII towards HindIII) and shows that ilvB regulation is at the level of transcription (data not shown). These results are consistent with the mRNA hybridization data of Newman et al. (7).

The 1.4 kb HindIII fragment from pCH2 was digested with restriction endonuclease

Table 2. The Combined Effect of Carbon Source and Leucine Starvation on AHAS Expression in Strain CU888leu20/pCH4

	Excess Leucine		Limiting Leucine	
Carbon Source and Additions	AHAS sp act	Relative sp act[a]	AHAS sp act	Relative sp act[a]
Glucose-6-Phosphate	70	0.6	490	4.4
Glucose	110	1.0	630	5.7
Glucose + cAMP	220	2.0	1140	10.4
Glycerol	220	2.0	950	8.6
Succinate	200	1.8	1360	12.4
Succinate + cAMP	200	1.8	1630	14.8

[a] Relative to sp. act. compared to a culture grown on glucose and excess leucine. Sp. act. in nmoles acetoin/min/mg protein. Cultures were grown to mid log in minimal media with 0.5% of the indicated carbon source. Where indicated 3mM cAMP was added 1.5 hr before assaying AHAS sp. act.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

Sau3A and cloned into the BamH1 site of plasmid pK011 (a derivative of pKO1 with a BamH1 site preceding the galK gene). A pKO11 plasmid containing a 650 bp Sau3A fragment, pCH3, which regulates galK expression in the same manner as ilvB in response to amino acid limitation was found. However, the expression of galactokinase on this fusion plasmid was no longer regulated by catabolite repression. Thus, the Sau3A fragment contains the ilvB promoter-attenuator region but has apparently lost the DNA sequences required to effect the catabolite repressor protein (CRP) cyclic AMP mediated regulation. This result also implies that the ilvB transcription start site should be near the galK distal end of the 650 bp Sau3A fragment in pCH3. A 280 bp Sau3A-HaeIII restriction fragment from this region of the pCH3 insert was cloned into M13 mp8 and M13 mp9 phage allowing the DNA sequence of both strands of this 280 bp fragment to be determined (Fig. 1).

DNA Sequence of the ilvB Promoter-Attenuator Region

The DNA sequence of the ilvB regulatory region contained in plasmid pCH3 is shown in Figure 1. The proposed transcription start site at base pair +1 is inferred from DNA sequence homology to consensus promoter sequences (23,24), as well as size determina-

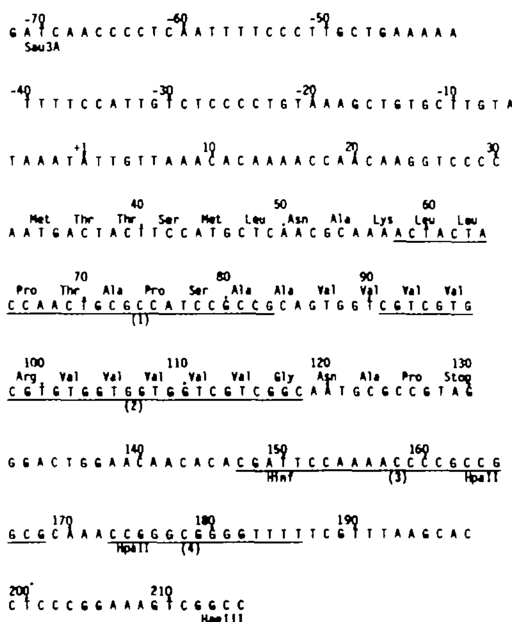

Figure 1 - DNA sequence of the ilvB regulatory region. The DNA sequence is numbered from the proposed point of transcription initiation. Underlined sections represent potential base pairing regions of the leader RNA shown in schematic representation in Figures 2, 4, and 5.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

tions of in vitro transcription products (Fig. 3). The base pairs -15 to -9 include a region for RNA polymerase function similar to that originally described by Pribnow (25). Further removed at base pairs -34 to -28 is a second sequence similar to sequences reported to be important for promoter function (24). Beginning at 183 bases from the proposed transcription start site is a string of thymidine residues preceded by a G + C rich region of dyad symmetry similar to other transcription termination sites (24). Indeed, an in vitro RNA transcript of 184 nucleotides is produced from the plasmid pCH3 (Fig. 3, lane 1). This putative leader RNA encodes a short leader polypeptide of 32 amino acids containing tandem codons for valine and leucine, amino acids involved in the multivalent repression of the ilvB operon. This leader RNA can form secondary structures typical of leader RNAs of other operons regulated by attenuation (Fig. 2). The free energy of these structures calculated by the method of Tinoco et al. (26) is shown in Table 3.

In Vitro Transcriptions

That transcription of the leader RNA of the ilvB attenuator initiates and terminates at the sites postulated above is demonstrated by the results of in vitro transcriptions of restriction fragments (Fig. 3). Transcription of the 283 base pair Sau3A-HaeIII fragment (Fig. 1) yields a 184 nucleotide transcript (Fig. 3, lane 5). Transcription of the shorter 235 base pair Sau3A-HpaII fragment (Fig. 1), which ends nineteen bases before the string

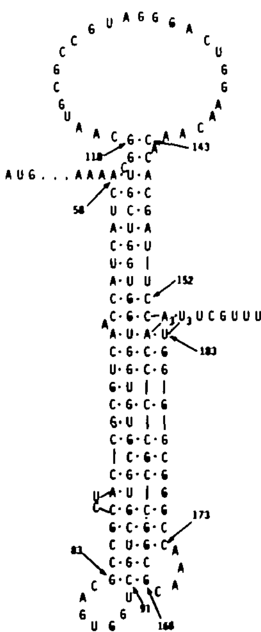

Figure 2 - A proposed secondary structure of the ilvB leader RNA.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

Table 3. Estimated Free Energies at 25°C of the Base-paired Regions of the ilvB Leader RNA

Base Paired Region	Nucleotides Base Paired	Δ G, Kcal
1 with 2	58-83 with 91-115	-16.7
2 with 3	91-118 with 143-168	-30.8
3 with 4	154-166 with 173-186	-33.2
B with C	61-74 with 80-93	-16.6
A with D	38-55 with 96-111	-15.2

of thymidine residues produces a 167 nucleotide transcript (Fig. 3, lane 4). Transcription of the still shorter 219 base pair Sau3A-Hinf fragment (Fig. 1) produces a 150 nucleotide transcript (Fig. 3, lane 3).

It is known that substituting ITP for GTP in in vitro RNA synthesis decreases the efficiency of transcription termination (27,28). Transcription of the 283 base pair Sau3A-HaeIII fragment in the presence of GTP produces the predicted 184 nucleotide leader RNA and a lesser amount of a 210 nucleotide runoff transcript. Substitution of ITP for GTP in this reaction results in the disappearance of the 184 nucleotide terminated leader transcript and the enhanced production of the 210 nucleotide transcript

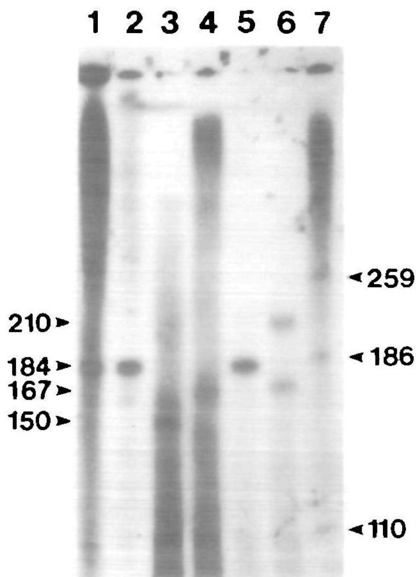

Figure 3 - In vitro transcription products of restriction fragments from the ilvB regulatory region. RNA transcribed from: lane 1, pCH2; lane 2, the 650 bp Sau3A fragment; lane 3, the 219 bp Sau3A-HinfI fragment; lane 4, the 235 bp Sau3A-HpaII fragment; lanes 5 and 6, the 283 bp Sau3A-HaeIII fragment using GTP and ITP respectively, lane 7, pAH29 (36) which encodes the ilvGEDA leader RNAs as size markers. The origin of the small transcript in lane 6 is unknown.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

(Fig. 3, lanes 5 and 6). This result is consistent with RNA polymerase reading through the terminator sequence to the end of the restriction fragment. The sequenced ilvGEDA leader transcripts (C. Adams, unpublished data) were included as size markers (Fig. 4, lane 7).

DISCUSSION

We report here the molecular cloning of the ilvB operon of E. coli K12 and the nucleotide sequence of an attenuator region of this operon. Examination of the expression of the ilvB operon cloned into multicopy plasmids show that the cloned ilvB gene is regulated by both amino acid limitation and catabolite repression as previously reported for the chromosome encoded gene (19,6). Enhanced expression of the ilvB gene product from plasmid pCH4 in a strain containing a hisT lesion (Table 1) suggests that regulation of this gene by amino acid limitation is due to an attenuator mechanism. Further evidence is presented which confirms that the expression of this cloned gene is also regulated by CRP cyclic AMP mediated catabolite repression (Table 2; 6). This result implies the existence of a CRP-cyclic AMP binding site in the ilvB regulatory region. Subcloning of the ilvB regulatory region of pCH4 produced a plasmid, pCH3, which retains regulation by amino acid limitation but loses regulation by catabolite repression. This plasmid contains a 650 base pair Sau3A restriction fragment which includes only 71 base pairs preceding the postulated transcription start site of the ilvB operon. The nucleotide sequence of this region (Fig. 1) shows no apparent homology to

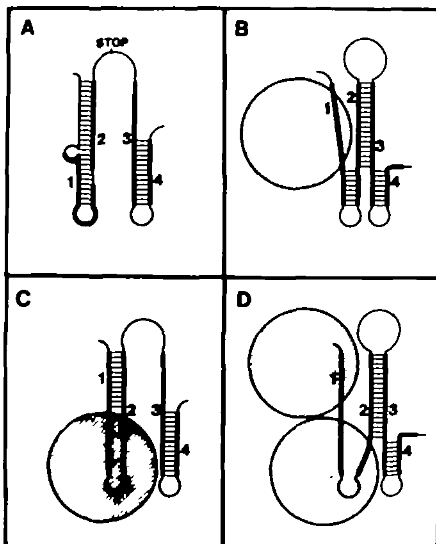

Figure 4 - Postulated effect of ribosome positioning on the secondary structure of the ilvB leader RNA. Black bars represent regions 1-4. Base pairing of these regions is schematically indicated. (A) Translation under nonlimiting condition; (B) ribosome stalled at tandem leucine codons; (C) ribosome stalled at the tandem valine codons; (D) ribosome stalled at the tandem valine codons with attachment of second ribosome.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

other CRP-cyclic AMP binding sites (29-32). It, therefore appears that a functional CRP-cyclic AMP binding site is not contained within the 71 base pairs preceding the transcription start site of this operon. This distance is greater than those observed for the lac or gal operons (29,30) but consistent with the -92 base pair location of the CRP-cyclic AMP binding site of the araBAD operon (31).

On the basis of our in vitro transcription data (Fig. 3), and by analogy to other transcription termination sites (24), we have identified a site that terminates a 184 nucleotide leader RNA transcribed from the ilvB regulatory region. The site of termination is located in a sequence of five thymidine residues proceeded by a G + C rich region of dyad symmetry (regions 3 and 4; Figs. 2 and 5). The RNA secondary structure that can form in this region is presumed to be the terminator stem-loop structure of the ilvB attenuator. Other regions capable of forming stable secondary structures include the 1-2 and 2-3 stem-loops shown in Figure 1 and 2. The structures shown in Figure 2 are mutually exclusive, only the 1-2 and 3-4 or 2-3 regions can base pair at any one time.

Examination of the DNA sequence in Figure 1 reveals a ribosome binding site followed by a potential coding region for a 32-amino acid leader polypeptide preceding the transcription termination site. The two tandem leucine codons and the four tandem valine codons in the putative leader peptide are positioned such that reduction of aminoacylation of the respective tRNAs should result in relief of attenuation (increased transcriptional readthrough into the structural gene) according to the current model of attenuation (33). In keeping with this model, regulatory interactions between ribosomes translating the leader peptide and the leader RNA secondary structure are proposed in Figure 4.

The model of regulation for other attenuators postulates the temporal formation of stem-loop 1-2 followed by the terminator stem-loop 3-4 during transcription in the absence of translation (33). However, during transcription of the ilvB leader RNA in the absence of translation, or under repressing conditions, an RNA secondary structure unique to this attenuator could form. Figure 5 shows that the early base pairing of regions B and C in region 1 followed by the subsequent base pairing of region D (most of region 2) with region A would preclude the formation of the 1-2 stem-loop. However, since region 2 is unavailable for base pairing with region 3, the 3-4 stem-loop can still form. Thus, the resultant RNA structure still would be expected to effect transcription termination in vitro which is shown to occur in Figure 3. The same structures should form under repressing conditions when the movement of ribosomes through the leader RNA is unimpeded (Fig. 4a). Another in vivo condition where stem-loop B-C could form, is under limitation for aminoacylated threonyl tRNA, where a ribosome pausing on the tandem threonine codons of the leader polypeptide at base pair 35 to 40 (Fig. 1), could prevent base pairings between regions A and D (Fig. 5). This would free region 2 to bind

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

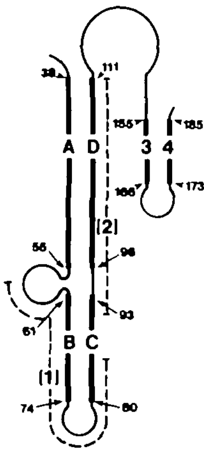

Figure 5 - Postulated secondary structure of the ilvB leader RNA in the absence of translation or translation under nonlimiting conditions. Black bars represent base paired regions. Two short stem-loops within the loop from bp 111 to 155 (bp 115-118 with bp 124-127 and bp 135-140 with bp 148-154) are not depicted in the schematic.

with region 3, leading to deattenuation. The in vivo affect of such a limitation is not presently known.

Since it has been reported that ribosomes mask about three codons 3' to the codon being read (34), pausing of ribosomes on the tandem leucine codons (bp 59-64; Fig. 1) of the leader polypeptide (under conditions of limiting aminoacylated leucyl-tRNA) would preclude the formation of the B-C stem-loop (Fig. 2) and prevent the 5' half of region 1 from base pairing with complementary sequences in region 2 (Fig. 5). This in turn would facilitate the partial formation of stem-loop 2-3 and inhibit the formation of the top portion of the 3-4 terminator stem-loop as shown in Figure 4b. This disruption of the terminator stem-loop would decrease its thermodynamic stability by nearly 60%. Disruption of base pairing in the terminator stem-loop to this extent should cause increased transcriptional readthrough (deattenuation).

The four tandem valine codons (Fig. 1) are situated in the leader polypeptide coding region such that ribosome pausing in this region due to limitation for aminoacylated valyl-tRNA would disrupt rather than promote base pairing in the 2-3 stem-loop, thereby facilitating the complete formation of the 3-4 terminator stem-loop (Fig. 4c). In order to account for deattenuation under these circumstances it is necessary to postulate the queueing of a second ribosome on the leader RNA. Pausing of the first ribosome should physically stall the second ribosome at the approximate location of the tandem leucine codons in region 1. This positioning of the second ribosome could then cause deattenuation in a manner similar to that described for leucine limitation (Fig. 4b and d). The necessity of loading a second ribosome in order to effect deattenuation also has been postulated for the ilvGEDA multivalent attenuator (12).

This model of attenuation of ilvB expression by leucine and valine predicts that the

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

3' half of region 3 will always be base paired to the 5' half of region 4, and that regulation will be effected only by disruption and formation of base pairings in the other half of the terminator stem-loop structure (Fig. 4). This model also suggests a mechanism which couples the extent of valine starvation to the level of deattenuation. It is reasonable to assume that the distance the ribosome travels through the tandem valine codons ascending region 2 (Figs. 1 and 2) will be inversely propotional to the availability of aminoacylated valyl-tRNA. The further the ribosome proceeds into region 2 the more it facilitates attenuation (stem-loop 3-4 formation). This means the more severe the valine limitation the greater the deattenuation. The regulatory significance, if any, of the five tandem valine codons (base pairs 101 to 115 in Fig. 1) is unknown.

Winkler and Yanofsky have recently reported the presence of an RNA polymerase transcription pause site near the translation stop codon of the trp attenuator leader polypeptide (35). They postulate that one function of this pause site is to insure proper coupling of transcription and translation for the regulation of the tryptophan attenuator. If this is a universal feature of attenuators and is of in vivo significance, such a pause site in the ilvB attenuator could help insure sufficient time for loading and positioning of a second ribosome as postulated in the two ribosome model of attenuation described above.

The RNA secondary structure shown in Figure 2 and the amino acid sequence of the leader polypeptide shown in Figure 1 predict that in vivo reduction of aminoacylated alanyl-tRNA should cause deattenuation of the ilvB operon. This is due to the position of tandem alanine codons in region 1 (Figs. 1 and 2). Ribosomes stalling on this set of tandem alanine codons would promote formation of the 2-3 stem-loop thereby inhibiting the formation of the 3-4 terminator stem-loop. At present the consequence of in vivo limitation of aminoacylated alanyl-tRNA on the expression of the ilvB operon is not known.

Note Added in Proof

Since the submission of this work, Friden et al. (37) have also described the DNA sequence of the ilvB attenuator. Their postulated leader RNA secondary structures are significantly different from those proposed here. Because of the temporal nature of RNA folding we believe that the RNA structures described in this report are more likely to form. Experiments designed to test the distinct predictions of each model are in progress.

ACKNOWLEDGMENTS

We thank Craig Adams for helpful suggestions and discussions. This work was supported in part by a grant (GM 24330) from the National Institutes of Health. C.A.H. was the recipient of a National Institutes of Health Predoctoral Training Grant (GM 07134).

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024

REFERENCES

1. Guardiola, J., DeFelice, M., Lamberti, A., and Iaccarino, M. (1977) Mol. Gen. Genet. 156, 17-25.
2. Favre, R., Wiater, A., Puppo, S., Iaccarino, M., Noelle, R., and Freundlich, M. (1976) Mol. Gen. Genet. 143, 243-252.
3. DeFelice, M. and Levinthal, M. (1977) Biochem. Biophys. Res. Commun. 79, 82-87.
4. Whitlow, K. J. and Polglase, W. J. (1974) FEBS Letters 43, 64-66.
5. Freundlich, M. (1977) Cell 12,1121-1126.
6. Sutton, A. and Freundlich, M. (1980) Mol. Gen. Genet. 178, 179-183.
7. Newman, T., Friden, P., Sutton, A., and Freundlich, M. (1982) Mol. Gen. Genet. 186, 378-384.
8. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory.
9. Stormer, F. and Umbarger, H. E. (1964) Biochem. Biophys. Res. Commun. 17, 587-592.
10. Bradford, M. M. (1976) Anal. Biochem. 72,248.
11. Sanger, F., Nicklen, S., and Coulsen, A. (1977) Proc. Natl. Acad. Sci USA 74, 5463-5467.
12. Lawther, R. and Hatfield, G. W. (1980) Proc. Natl. Acad. Sci. USA 77, 1862-1866.
13. Newman, T. and Levinthal, M. (1979) Genetics 96, 59-77.
14. Clarke, L. and Carbon, J. (1976) Cell 9, 91-99.
15. Subrahmanyam, C. S., McCorkel, G., and Umbarger, H. E. (1980) J. Bact. 142, 547-555.
16. Chang, A., Chang, C. Y., and Cohen, S. (1978) J. Bact. 134, 1141-1156.
17. De Felice, M., Levinthal, M., Iaccarino, M., and Guardiola, J. (1979) Microb. Rev. 43, 42-58.
18. Lawther, R. P. and Hatfield, G. W. (1977) J. Bact. 130, 552-557.
19. DeFelice, M., Newman, T., and Levinthal, M. (1978) Biochem. Biophys. Acta 541, 1-8.
20. Roth, J., Anton, D., and Hartman, P. (1966) J. Mol. Biol. 22, 305-323.
21. Cortese, R., Landsberg, R., Vonder Haar, R., Umbarger, H. E., and Ames, B. (1974) Proc. Natl. Acad. Sci. USA 71, 1857-1861.
22. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C., and Rosenberg, M. (1981) in Gene Amplification and Analyses, Vol. II, Holland, E., Chrikjian, J., and Papas, T. Eds.
23. Scherer, G., Walkinshaw, D., and Arnot, S. (1978) Nucleic Acids Res. 5, 3759-3773.
24. Rosenberg, M. and Court, D. (1979) Annu. Rev. Genet. 13, 319-353.
25. Pribnow, D. (1975) Proc. Natl. Acad. Sci. USA 72, 784-788.
26. Tinoco, I., Borer, P., Dengler, B., and Lavine, M. (1973) Nature New Biol. 246, 40-41.
27. Lee, F. and Yanofsky, C. (1977) Proc. Natl. Acad. Sci. USA 74, 4365-4369.
28. Neff, N. and Chamberlin, M. (1978) J. Biol. Chem. 253, 2455-2460.
29. Reznikoff, W. and Abelson, J. (1978) in The Operon, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
30. deCrombugghe, B. and Pastan, I. (1978) in The Operon, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
31. Ogden, S., Haggerty, D., Stoner, C., Koladrubetz, D., and Schleif, R. (1980) Proc. Natl. Acad. Sci. USA 77, 3346-3350.
32. Deeley, M. Yanofsky, C. (1981) J. Bact. 147, 787-796.
33. Yanofsky, C. (1981) Nature 289, 751-758.
34. Oxender, D., Zurawski, G., and Yanofsky, C. (1979) Proc. Natl. Acad. Sci. USA 76, 5524-5528.
35. Winkler, M. and Yanofsky, C. (1981) Biochemistry 20, 3738-3744.
36. Lawther, R., Calhoun, D., Adams, C., Hauser, C., and Hatfield, G. W. (1981) Proc. Natl. Acad. Sci. USA 78, 922-925.
37. Friden, P. Newman, T., and Freundlich, M. (1982) Proc. Natl. Acad. Sci. USA 79, 6156-6160.

Downloaded from https://academic.oup.com/nar/article/11/1/127/1040067 by guest on 24 April 2024