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The DNA sequence of the promoter-attenuator of the ilvGEDA operon of Salmonella typhimurium

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ABSTRACT

The isolation of a λgt·ilvGEDA·S.t. hybrid transducing phage has permitted the characterization of the promoter-attenuator region of the ilvGEDA operon of Salmonella typhimurium. In vitro transcription and Southern hybridization indicate that the promoter-attenuator resides on a 400 nucleotide Rsa I restriction fragment. DNA sequence analysis shows only seven base pair differences exist between the DNA sequence of the ilvGEDA promoter-attenuator of S. typhimurium and that previously published for Escherichia coli K12.

INTRODUCTION

Four of the genes for the biosynthesis of isoleucine and valine form the ilvGEDA operon in both Escherichia coli K12 (3,13,17,18) and Salmonella typhimurium (2,5,27). In both organisms, the three amino acids isoleucine, leucine, and valine regulate this operon by multivalent repression as a function of the percent aminoacylation of their respective transfer RNAs (reviewed in ref. 6). Transcription in vitro and DNA sequence analysis (17, 18,22) indicate that the ilvGEDA operon of E. coli K12 is regulated by attenuation in a manner similar to that proposed for other systems regulated by transfer RNA (14,15,23). The in vitro transcription of plasmids and DNA restriction fragments yields two leader-like RNAs of 180 and 250 nucleotides. DNA sequence analysis of this transcribed region indicates a possible 32 amino acid peptide that would contain four leucine, five isoleucine and six valine residues. Also, the DNA sequence implies that the RNA transcripts would form the secondary structures required for attenuation.

To gain further insight into the regulation of the ilvGEDA operon and to increase understanding of attenuation, the regulatory region of the ilvGEDA operon of S. typhimurium was analysed. In vitro transcription of

the regulatory region yields a 180 nucleotide transcript, i.e. probable leader RNA. DNA sequence analysis indicates that within the region from 46 base pairs prior to the estimated start of transcription to the termination site (232 base pairs), only seven base pair differences exist between the regulatory region of S. typhimurium and E. coli K12.

MATERIALS AND METHODS

(a) Materials

Restriction endonucleases either were obtained from New England Biolabs or were the generous gift of either Dr. G. W. Hatfield or Dr. J. Gardner (Rsa I). E. coli DNA-dependent RNA polymerase was obtained from New England Biolabs. Bacterial alkaline phosphatase was obtained from Worthington Biochemical Corp. and T4 polynucleotide kinase was obtained from P. L. Biochemicals, Inc. T4 DNA ligase was obtained from Bethesda Research Laboratories, Inc.

(b) Preparation of plasmids and λ phage DNA

Plasmid DNA was prepared as previously described (18) and λ phage was propagated and purified as described by Davis, et al. (9). DNA was extracted from the phage by treatment with 0.1 mg/ml proteinase K in 0.1% SDS, 10mM $MgCl_2$ 10mM Tris-HCl, pH 7.5 for 30 minutes at 37°C. Subsequently, the DNA was extracted twice with an equal volume of redistilled phenol (presaturated with 10mM Tris-HCl, pH 7.5), and then once with a 1:1 mixture of phenol and chloroform and finally extracted with chloroform. The DNA was dialyzed against two one liter changes of 20mM EDTA, pH 8.0 and two one liter changes of 1mM EDTA, 10mM Tris-HCl pH 8.0.

(c) In vitro transcription and hybridization

In vitro transcription reactions were performed as described previously (17, 18). Each reaction contained 0.1 pmole of DNA template and 0.5 pmole E. coli DNA-dependent RNA polymerase. After termination, the reactions were subjected to electrophoresis on a 7M urea 6% polyacrylamide gel (18). For preparation of in vitro transcripts as a hybridization probe, the transcripts were visualized by autoradiography and then excised and eluted from the gel as described by Maxam and Gilbert for DNA restriction fragments (20). Southern (29) transfer of DNA restriction fragments and hybridization of the in vitro transcripts was by the method of Davis, et al. (9).

(d) DNA sequencing

Preparation of DNA restriction fragments and DNA sequence analysis were performed as described by Maxam and Gilbert (20).

RESULTS

(a) Isolation of λgt7·ilvGEDA·S.t.

Complementation of an E. coli K12 ilvA$^-$ mutant permitted the isolation of a hybrid λ phage that contains the ilvGEDA operon of S. typhimurium. Coinfection of a λgt7·S.typhimurium Eco RI pool (9) into CU406 (ilvA454, ref. 28) with λgt4·K (9) as a helper phage yielded ILV$^+$ transductants when plated on M63 minimal media (21). Because of the cryptic nature of ilvG (see 10 & 6, but also 3), valine inhibits the growth of E. coli K12 but does not inhibit the growth of S. typhimurium which contains a functional ilvG gene (10). Thus the growth of the ILV$^+$ transductants, when transferred to M63 media containing 1mM valine, indicated the presence of the ilvG gene of S. typhimurium. Since the transductants contain the first gene (ilvG, VAL^R) and the last gene (ilvA, ILV$^+$) of the ilvGEDA operon from S. typhimurium, it follows that they contain the entire operon; this has been confirmed by further genetic analysis (unpublished observations M.P. Taillon and R.P. Lawther). Thermal induction of one of the ILV$^+$ transductants with subsequent plaque purification yielded λgt7·ilvGEDA·S.t..

(b) Construction of pRL103

Digestion of λgt7·ilvGEDA·S.t. with the restriction endonuclease Eco RI yields three restriction fragments, the 19.6 and 15.2 kilobase (Kb) arms of λgt7 (9) and an 11 Kb insert (Figure 1A, lane 2). This indicates that the entire ilvGEDA operon of S. typhimurium is probably on a single Eco RI restriction fragment. Digestion of λgt7·ilvGEDA·S.t. with Hind III yields six restriction fragments (Figure 1A, lane 3). To establish which of these fragments might contain the regulatory region of the S. typhimurium ilvGEDA operon, the 180 nucleotide leader RNA of E. coli K12 (17,18,23) was hybridized to a Southern blot (9,29) of the restriction digest presented in Figure 1A. Examination of Figure 1B (lanes 2 and 3) shows that the E. coli leader RNA hybridizes with the 11 Kb insert of the phage (lane 2) and the 5.5 Kb Hind III restriction fragment (lane 3).

Insertion of the 5.5 Kb Hind III restriction fragment into the Hind III site of pBR322 yielded pRL103. That this plasmid contains only the 5.5 Kb insert can be seen by comparison of lanes 3, 4 and 5 of Figure 1A. Digestion of pRL103 with Hind III (lane 5) results in a pair of restriction fragments, the 5.5 Kb Hind III fragment from the phage (lane 3) and the single fragment from pBR322 (compare with lane 4). Complementation and restriction analyses (unpublished observations M.P. Taillon and R.P. Lawther) indicate

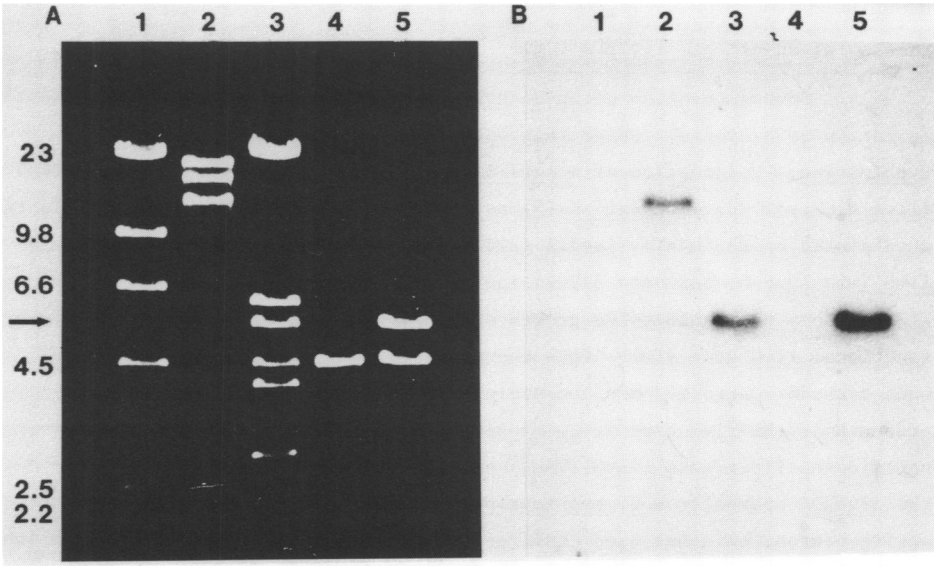

Figure 1A - Agarose gel electrophoresis of Hind III restriction endonuclease digestions of λ (lane 1), λgt7·ilvGEDA·S.t. (lane 3), pBR322 (lane 4) and pRL103 (lane 5) and an Eco RI digestion of λgt7·ilvGEDA·S.t. (lane 2). The DNA was digested with the indicated restriction endonuclease and subjected to electrophoresis on an 0.8% agarose gel in 100mM Tris-acetate pH 8.05, 40mM sodium acetate, and 4mM EDTA at 25 volts for 16 hours. Fragment lengths in Kilobases (Kb) for λ are from Davis, et al. (9) and the arrow indicates 5.5 Kb.

Figure 1B - Autoradiograph of the 180 nucleotide leader RNA of the ilvGEDA operon of E. coli K12 (prepared in vitro) hybridized to a Southern blot (9,29) of the agarose gel presented in Figure 1A.

that the plasmid contains ilvG and ilvE, which are transcribed in the opposite direction of the genes for tetracycline resistance (Figure 2).

(c) In vitro transcription of pRL103 and the localization of the ilvGEDA promoter-attenuator.

The transcription in vitro of the plasmid pRL5 which contains the promoter-attenuator region of the E. coli K12 ilvGEDA operon results in the formation of a 180 and a 250 nucleotide transcript (Figure 3, lane 2; ref. 17,18). Therefore pRL103 was transcribed to ascertain the size and number of transcripts from the S. typhimurium ilvGEDA operon. Figure 3 is an autoradiograph of the products of the transcription in vitro of pBR322 (lane 1), pRL5 (lane 2) and pRL103 (lane 3). Two major transcripts are

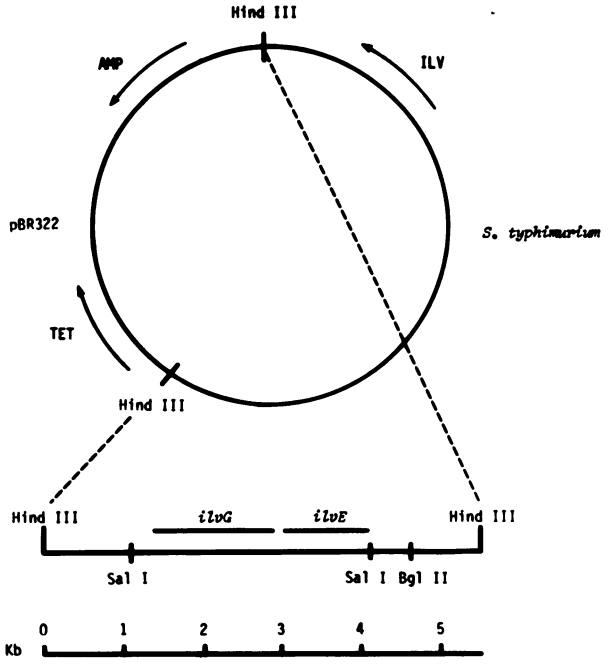

Figure 2 - Restriction endonuclease map of the 5.5 Kb Hind III restriction fragment contained in pRL103. The direction of transcription of the genes for ampicillin resistance (AMP), tetracycline resistance (TET) and for isoleucine and valine biosynthesis are indicated by arrows (30). The 5.5 Kb Hind III restriction fragment contains no restriction sites for the restriction endonucleases: BAM HI, Eco RI, Kpn I, Pst I, Pvu II, Xho I, and Xba I.

present in lane 3 (pRL103) - one is an approximately 110 nucleotide RNA transcript that originates from pBR322 (lane 1) and the second is an 180 nucleotide RNA similar to the smaller of the two E. coli K12 RNAs (lane 2). Three lines of evidence indicate that the 180 nucleotide transcript originates from the S. typhimurium regulatory region. First, the 5.5 Kb Hind III restriction fragment of pRL103 hybridizes with the E. coli K12 180 nucleotide leader RNA (Figure 1B, lane 5). Second, the plasmid pRL103 expresses ilvGE, while the orientation of these genes makes it unlikely that their expression originates from a plasmid promoter (Figure 2). Finally, the S. typhimurium transcript (Figure 3, lane 3) is essentially identical in size to that of E. coli K12 (Figure 3, lane 2).

Hybridization of the S. typhimurium 180 nucleotide RNA (synthesized in

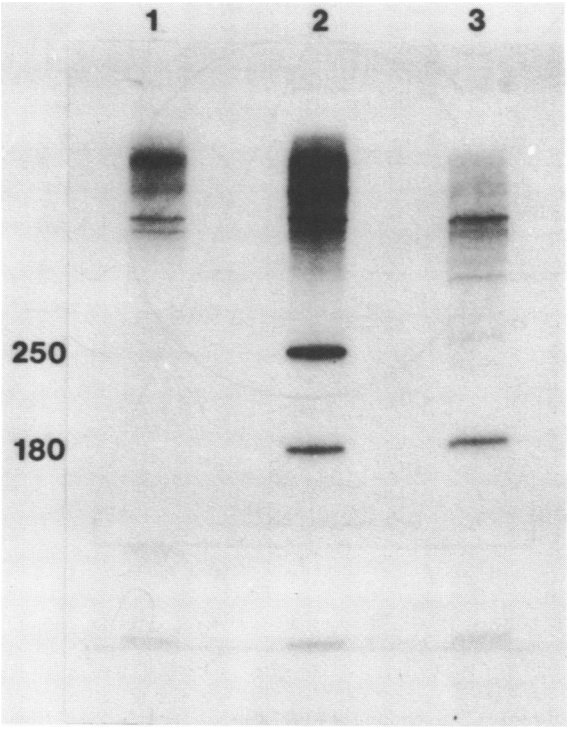

Figure 3 - Autoradiograph of products of the transcription in vitro of pBR322, pRL5, and pRL103. Transcription was as described in Materials and Methods, and previously (17,18). Lane 1 contains the products of the transcription in vitro of pBR322. Lane 2 contains the products of the transcription of pRL5. Lane 3 contains the products of the transcription of pRL103. The plasmid pRL5 is a derivative of pBR322 that contains the promoter-attenuator and ilvG of E. coli K12 (18).

vitro) with restriction digests of pRL103 permitted the localization of the origin of this transcript. A Southern blot (29) of a series of individual restriction endonuclease digests (Alu I, HinF I, Rsa I, and Sau3A I; Figure 4A) of pRL103 was hybridized with the 180 nucleotide transcript of pRL103. Figure 4B is an autoradiograph of this hybridization and, as can be seen, the 180 nucleotide transcript hybridizes principally to the larger restriction fragments in each digest (i.e. 1 Kb or greater). The exception to this is the Rsa I digest (Figure 4B, lane 4), where the RNA hybridizes to one of a pair of restriction fragments which are approximately 420 nucleotides in length. This experiment lacks sufficient resolution

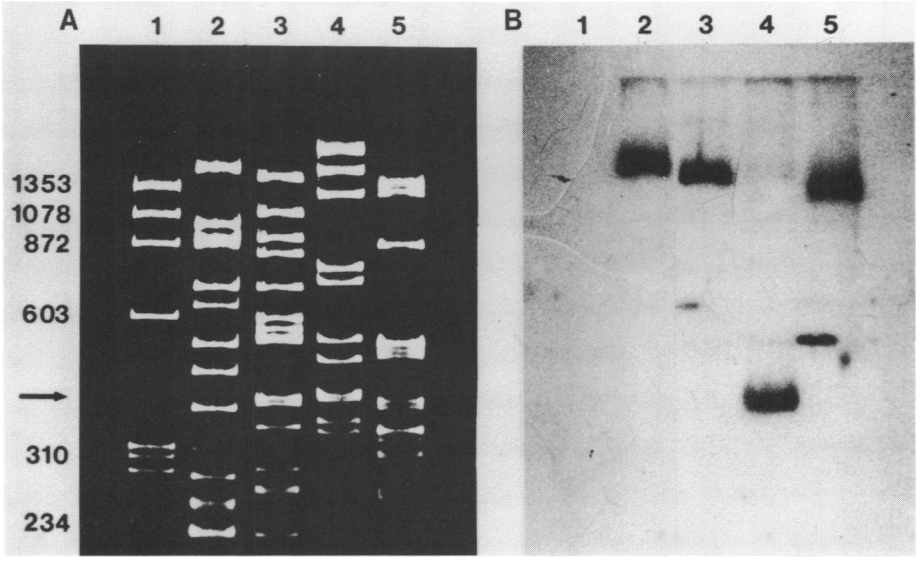

Figure 4A - Agarose-acrylamide gel electrophoresis of restriction endonuclease digests of pRL103. The plasmid was digested with:Alu I (lane 2), HinF I (lane 3), Rsa I (lane 4), and Sau3A I (lane 5). Lane 1 is a Hae III restriction endonuclease digestion of ØX174. Restriction fragment lengths in base pairs (bp) are per Fuchs, et al. (12) and the arrow indicates 420 bp. The restriction digests were subjected to electrophoresis on an 0.8% - 6% polyacrylamide gel as described by Reiser, et al. (24).

Figure 4B - Autoradiograph of the 180 nucleotide leader RNA of the ilvGEDA operon of S. typhimurium (prepared in vitro) hybridized to a Southern blot (9,29) of the agarose-polyacrylamide gel presented in Figure 4A.

to determine which of the two Rsa I restriction fragments hybridizes the transcript.

Transcription in vitro established that the 180 nucleotide transcript originates from the smaller of the two Rsa I restriction fragments (Figure 5). To serve as templates for in vitro transcription, the two Rsa I restriction fragments were prepared as described by Maxam and Gilbert (20). Figure 5 is an autoradiograph of the products of the transcription in vitro of pRL103 (lane 1), the larger Rsa I restriction fragment (lane 2), and the smaller Rsa I restriction fragment (lane 3). Only transcription of the smaller Rsa I restriction fragment yields the 180 nucleotide transcript. This demonstrates that this Rsa I restriction fragment contains

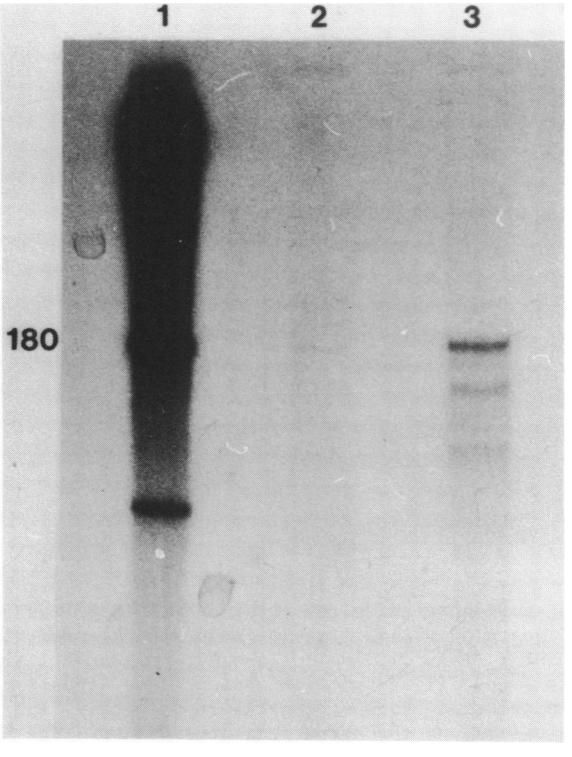

Figure 5 - Autoradiograph of the products of the transcription in vitro of pRL103 and the Rsa I restriction fragments. Transcription was as described in Materials and Methods, and previously (17, 18). Lane 1 contains the products of transcription in vitro of pRL103. Lane 2 contains the products of the transcription of the larger Rsa I restriction fragment. Lane 3 contains the products of the transcription of the smaller Rsa I restriction fragment.

all the necessary information for the initiation and termination of RNA synthesis, yielding the 180 nucleotide transcript.

(d) DNA Sequence

Having established that the 180 nucleotide transcript originates from the Rsa I restriction fragment, the DNA sequence was determined. Figure 6 presents the DNA sequence of the mRNA-like strand of the promoter-attenuator of the ilvGEDA operon of S. typhimurium. The bases beneath each line of the sequence are the bases at the corresponding positions in E. coli K12. The bases are numbered from the presumed start of transcription of this region, which is the same as that predicted for E. coli K12 (17,22) and which

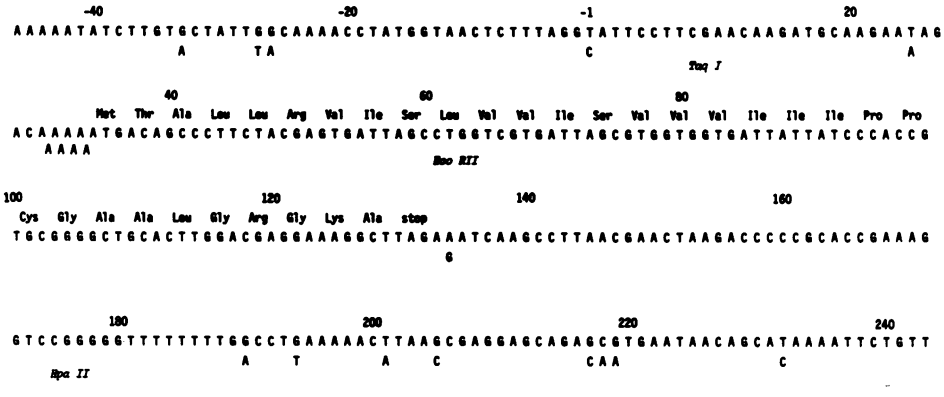

Figure 6 - DNA sequence of the regulatory region of the ilvGEDA operon of S. typhimurium. The sequence is numbered arbitrarily from the proposed points of transcription initiation. The bases beneath each line of the sequence are the bases at corresponding positions of this region in E. coli K12 (17).

has recently been confirmed for E. coli K12 by RNA fingerprint analysis (personal communication, C.W. Adams and G.W. Hatfield).

DISCUSSION

Previous analysis of the genetic structure and the regulatory properties of the ilvGEDA operon in S. typhimurium (2,5,7) and E. coli K12 (3,13,18) indicate a high degree of similarity in the organization and expression of this operon. The present experiments extend this relationship to near identity for the regulatory region of this operon. In vitro transcription of the promoter-attenuator region of both S. typhimurium (Figure 3, lane 3) and E. coli K12 (Figure 3, lane 2; ref. 17,22) yield a 180 nucleotide transcript. Further the DNA sequence of the promoter-attenuator of S. typhimurium (Figure 6) differs by only 7 of 235 base pairs from that of E. coli K12 (Figure 6; ref. 17,22). The conservation of the nucleotide sequence of this region has not only insured the preservation of the secondary structure of the leader RNA (as elaborated for the E. coli K12 sequence in references 17 & 18,22) but also extends to maintaining the codon choices of the presumed leader peptide.

Four of the seven differences found between the DNA sequence of the regulato: regions (Figure 6, base pairs - 46 to +189) of S. typhimurium and E. coli K12 occur within the promoter region (Figure 6, base pairs - 46 to +2). Comparison

of the DNA sequence of the ilvGEDA promoter of each organism to the promoter analyses of Rosenberg and Court (25) and of Siebenlist, et al. (26) predicts that the differences that exist between S. typhimurium and E. coli K12 would not result in a different level of expression of this operon in the two organisms. Proceeding from position -46 towards the start of transcription at +1, there is a difference at -33. In S. typhimurium this nucleotide is a G, while in E. coli K12 it is an A. Neither of these bases is the apparently preferred nucleotide (C is found for 36 of 54 sequenced promoters; ref. 26), although A predominates over G in the promoters that have been analysed. The next differences in the sequence are at -27 and -26. Analysis of the frequency distribution of the nucleotides of other promoters at these positions (25, 26) indicates that there is no preference for a particular base pair at either of these positions. The final difference is at -1 where the E. coli sequence contains the preferred C, while S. typhimurium has the next most preferred base, T.

The remaining three nucleotide differences in the regulatory region of S. typhimurium and E. coli K12 occur in the transcribed portion. The first difference is at position +25 which is a T in S. typhimurium and an A in E. coli K12. The second difference in the transcribed portion of the regulatory region is an additional A between positions +30 and +34 (i.e. five A's in S. typhimurium and four A's in E. coli K12). Both of these nucleotides are proximal to the secondary structure proposed from the E. coli K12 sequence (Figure 7; ref 17, 22) that is required for attenuation (14, 15, 23). The last difference is at position +133 which is a difference of A for S. typhimurium and G for E. coli K12. This nucleotide is in a loop of the secondary structure (17, 22) of the leader RNA and distal to the region that is presumed to be translated (Figure 7). These differences presumably effect neither the amino acid sequence of the leader peptide nor the stability of the RNA secondary structure.

The identification of the S. typhimurium regulatory region depended on there being a high degree of homology between the regulatory regions of S. typhimurium and E. coli K12. Nevertheless, the degree of sequence equivalence of the ilvGEDA promoter-attenuator from these two organisms seems remarkable. Based upon heterologous hybridization studies of genomic DNA from S. typhimurium and E. coli K12, the sequences of these organisms appear to be only 40-50 percent related (7). However, specific genomic segments may vary from this general estimate as indicated by the comparison of the regulatory sequences of the tryptophan (trp) and histidine (his) operons. Published DNA sequences of the his attenuator region of S. typhimurium (1) and E. coli K12 (11) indicate a high degree of homology. Out of 153 base pairs, only eight base pair

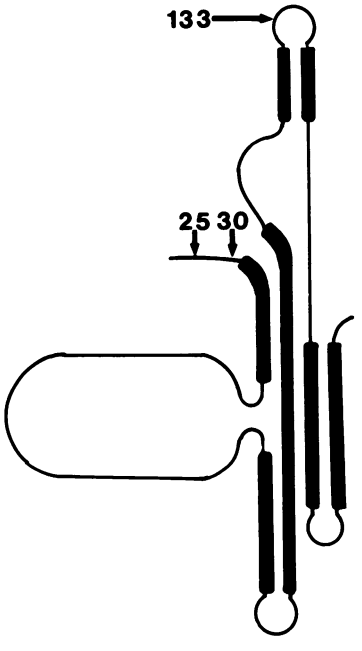

Figure 7 - Schematic representation of the proposed secondary structure of the ilvGEDA leader RNA. The positions of those nucleotides that differ between S. typhimurium and E. coli K12 are numbered as shown in Figure 6. The regions of nuceotide base-pairing are indicated by the heavy line.

differences are observed. None of these changes affect the proposed secondary structure of the attenuator region nor do they affect the nucleotide sequence of the region that encodes the presumptive leader peptide. Analysis of the trp promoter-operator-attenuator region (19) shows a much lower degree of homology with 73 base pair differences (in a total of 186). Seven of these differences are within the trp promoter-operator, and the remainder are within the trp attenuator. Thus, to what degree regulatory sequences are conserved in S. typhimurium and E. coli K12 is unclear, since the comparison of the three available sequences (his, trp, and ilvGEDA) indicates that the degree of conservation varies among specific genes. Further the remarkable specificity of the conservation of the promoter-attenuator of the ilvGEDA operon between S. typhimurium and E. coli K12 is illustrated by a comparison of the DNA sequence adjacent to the promoter in the opposite direction of transcription (Figure 8). In this region (-47 to -86) 26 of 40 bases are different, i.e. no conservation of this region is apparent.

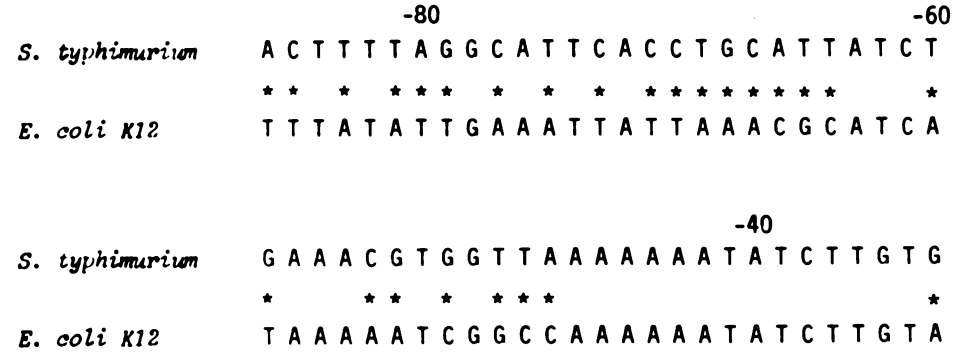

Figure 8 - DNA sequence of the region adjacent to the promoter-attenuator of S. typhimurium and E. coli K12. The sequence is numbered from the proposed point of transcription initiation (see Figure 6). The asterisks (*) indicate those nucleotides which differ between S. typhimurium and E. coli K12.

The reason for such strong retention of the DNA sequence of the ilvGEDA promoter-attenuator is not clear. One obvious constraint is the necessity of preserving the RNA secondary structure through base pairing. This appears not to be the full explanation since the sequence is preserved in regions not believed to participate directly in the secondary structure of the RNA (such as the loops between base paired regions). Because multiple codons exist for nearly all of the amino acids present in the putative leader polypeptide (Fig. 6; ref. 17,22), a need to maintain the amino acid sequence does not explain the retention of the nucleotide sequence either. From a comparison of the DNA sequence of the trpBA region of S. typhimurium and E. coli K12, Crawford, et al. (8) estimated the probability of third position substitutions in these structural genes is 0.5. They then calculated the probability of finding a 60 base sequence with no third base substitutions is 10^{-6}. By analogy the probability of finding the 90 base pair putative leader polypeptide region with no third base differences should be 10^{-9}. This may indicate a specific relationship between specific isoacceptor t-RNAs and the attenuator, or that our understanding of the mechanism of attenuation remains incomplete.

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