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NOTES

High A+T Content Conserved in DNA Sequences Upstream of *leuABCD* in *Escherichia coli* and *Salmonella typhimurium*

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The nucleotide sequence of over 800 base pairs of DNA upstream of *leuP* was determined for *Escherichia coli* and *Salmonella typhimurium*. In both of these enteric bacteria, approximately 500 base pairs of A+T-rich sequence separates *leuP* from an upstream open reading frame. Although these A+T-rich sequences share little homology, the distribution of A+T base pairs within the region is strikingly conserved. Deletion of the A+T-rich sequences upstream of the *E. coli leu* operon does not markedly affect the strength of the *leu* promoter in vivo.

The DNA of the *Escherichia coli* chromosome has an overall A+T content of approximately 49% (20). Nucleotide sequencing data indicate that those regions of *E. coli* chromosomal DNA which encode polypeptides generally have an A+T content equal to or lower than this estimate. For example, of 20 randomly chosen *E. coli* genes ranging in size from 234 to 1,839 base pairs (bp), the average A+T content was 47.6% with *lacY* (5) scoring the highest (54%). In contrast, A+T percentages of 65% or higher have been reported for sequences of 100 bp or more lying upstream of promoters of *E. coli* and its bacteriophages (6, 10, 12, 19). Several investigators have postulated that such A+T-rich sequences increase the strength of nearby promoters (6, 12), although to date no such role has been clearly demonstrated.

We report here an unusually extensive region of A+T-rich sequence upstream of the *leuABCD* operon of both *E. coli* (29) and *Salmonella typhimurium* (8, 9). Over 800 bp of DNA sequence upstream of the *leu* promoters of these organisms was determined (Fig. 1). As much as 550 bp of the DNA upstream of *leuP* is strikingly rich in A+T. For example, the sequence from position −83 to −583 is 71% A+T in *E. coli* and 69% A+T in *S. typhimurium*. By contrast, the 300 bp on either side of the A+T-rich region have a more typical base composition of about 50% (Fig. 1) (8, 29).

The distribution of A · T base pairs within several parts of the *leu-ilvIH* regions was diagrammed (Fig. 2). A+T percentages were calculated over 20-bp intervals centered at the position of the sequence indicated on the abscissa. Within the *ilvI* gene of *E. coli*, A+T-rich sequences are, in general, short and distributed in a manner similar to what would be found in a random sequence containing 49% A+T (Fig. 2A). The same pattern was observed for a part of *E. coli leuA* together with the associated promoter and leader regions. Within the A+T-rich region (positions −60 to −600), A+T-rich sequences were distributed as four or five peaks having A+T contents of 75 to 90% (Fig. 2B). A similar analysis of the comparable region of the *S. typhimurium* genome shows essentially the same pattern of A+T distribution. Note in particular that the peaks of high A+T content occur at the same positions for both organisms (Fig. 2E).

It might be expected that the similar A+T content patterns for *E. coli* and *S. typhimurium* are a reflection of sequence homologies within the regions compared. This is true for comparisons of the *leu* operon, but surprisingly it is not the case for the region of high A+T content. We compared sequence homologies by a graphic matrix analysis (14) using a window of 10 and a stringency of 0.80 (Fig. 3). The strong homology between the *leu* operons was apparent, whereas the A+T-rich sequences showed little sequence homology (Fig. 2D and 3).

The sequences upstream of *leu* were searched for ORFs. Between positions −50 and −450, stop codons are numerous in all three reading frames on both strands. The longest polypeptide with an N-terminal methionine that could be encoded by the A+T-rich sequences was 41 amino acids in *S. typhimurium* (Fig. 1B, positions −402 to −457) and 22 amino acids in *E. coli* (Fig. 1A, positions −368 to −433). It may be noted that to the left of the A+T-rich region, an ORF exists in both organisms (Fig. 1A, positions −457 to −855; Fig. 1B, positions −561 to −854). These ORFs have normal A+T contents (Fig. 2B and E) and share sequence homologies (Fig. 3). It is likely that the ORFs represent a heretofore unidentified gene that is transcribed in the same direction as is *ilvIH*. Additional sequencing results have shown that the *E. coli* ORF could encode a polypeptide containing 349 amino acids (G. Haughn and C. Squires, unpublished data).

To summarize, an A+T-rich region of about 550 bp exists between the *leu* operon and an ORF located clockwise to *leu* on the chromosome. This A+T-rich region probably does not encode a protein. Comparison of the A+T-rich regions of *E. coli* and *S. typhimurium* revealed a striking similarity between frequency and distribution of A · T despite extensive primary sequence divergence. Such a result was unlikely if sequence divergence occurred by random bp

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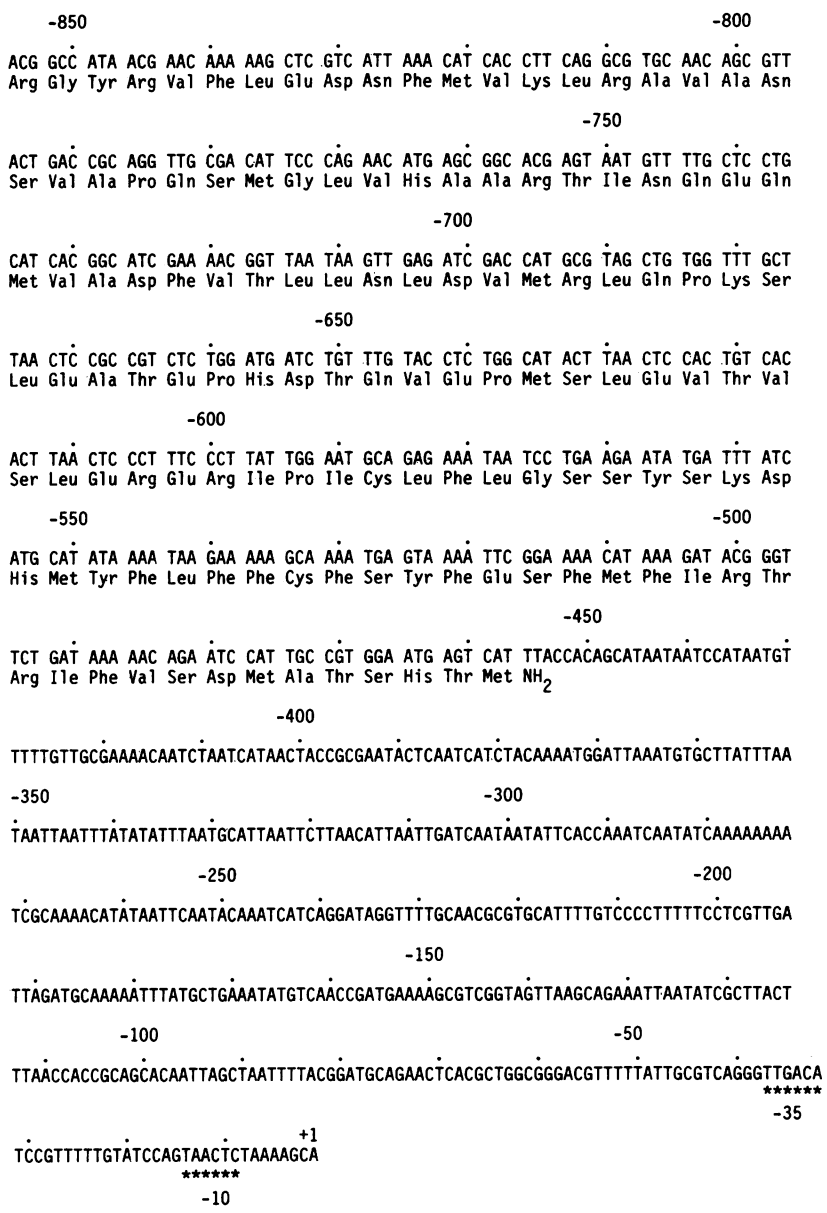
A
-850
-800
ACG GCC ATA ACG AAC AAA AAG CTC GTC ATT AAA CAT CAC CTT CAG GCG TGC AAC AGC GTT
Arg Gly Tyr Arg Val Phe Leu Glu Asp Asn Phe Met Val Lys Leu Arg Ala Val Ala Asn
-750
ACT GAC CGC AGG TTG CGA CAT TCC CAG AAC ATG AGC GGC ACG AGT AAT GTT TTG CTC CTG
Ser Val Ala Pro Gln Ser Met Gly Leu Val His Ala Ala Arg Thr Ile Asn Gln Glu Gln
-700
CAT CAC GGC ATC GAA AAC GGT TAA TAA GTT GAG ATC GAC CAT GCG TAG CTG TGG TTT GCT
Met Val Ala Asp Phe Val Thr Leu Leu Asn Leu Asp Val Met Arg Leu Gln Pro Lys Ser
-650
TAA CTC CGC CGT CTC TGG ATG ATC TGT TTG TAC CTC TGG CAT ACT TAA CTC CAC TGT CAC
Leu Glu Ala Thr Glu Pro His Asp Thr Gln Val Glu Pro Met Ser Leu Glu Val Thr Val
-600
ACT TAA CTC CCT TTC CCT TAT TGG AAT GCA GAG AAA TAA TCC TGA AGA ATA TGA TTT ATC
Ser Leu Glu Arg Glu Arg Ile Pro Ile Cys Leu Phe Leu Gly Ser Ser Tyr Ser Lys Asp
-550
-500
ATG CAT ATA AAA TAA GAA AAA GCA AAA TGA GTA AAA TTC GGA AAA CAT AAA GAT ACG GGT
His Met Tyr Phe Leu Phe Phe Cys Phe Ser Tyr Phe Glu Ser Phe Met Phe Ile Arg Thr
-450
TCT GAT AAA AAC AGA ATC CAT TGC CGT GGA ATG AGT CAT TTACCACAGCATAATAATCCATAATGT
Arg Ile Phe Val Ser Asp Met Ala Thr Ser His Thr Met NH2
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-50
TTAACCACCGCAGCACAATTAGCTAATTTTACGGATGCAGAACTCACGCTGGCGGGACGTTTTTATTGCGTCAGGGTTGACA
******
-35
+1
TCCGTTTTTGTATCCAGTAACTCTAAAAGCA
******
-10
B
-850
-800
AAT TCG TCC ATA TCG AAC AAA AAG TAC GTC ATT AAA CAT AAC CAC CAG ACG CGC TAC GGC
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Asn Ser Val Ala Pro Gln Ser Met Gly Leu Thr His Ala Ala Arg Thr Ile Asn Gln Glu
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Gln Met Val Ala Asp Phe Val Thr Leu Leu Asn Leu Asp Val Met Arg Leu Gln Pro Lys
-650
GCC CAT ATC TAA AAG ATG CGG CTT TCG GTT TTG ACC TCT GGC ATA TTT AAC TCC ACT GTC
Gly Met Asp Leu Leu His Pro Lys Arg Asn Gln Gly Arg Ala Tyr Lys Val Gly Ser Asp
-600
ACG CTT AAC TCC CTT TTT CTG GCT GAA GGC AGA AAT AAT TCC TGA AAA TAT GAT TTACCA
Arg Lys Val Gly Lys Lys Gln Ser Phe Ala Ser Ile Ile Gly Ser Phe Ile Ile NH2
-550
-500
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-250
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AATCAAAAAGATAAACCAAACCTTTAGCATACCTTTAACAGGCCATTCCCCTAACCAATTTGCAAACAAAAATGCC
-150
-100
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-50
TTAGCTAAAGTACGTATCCGGATATCGTCAACAAAATGCAATGGCGACAGAAAATAGAGTTGACATTAAACGGCAT
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+1
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******
-10

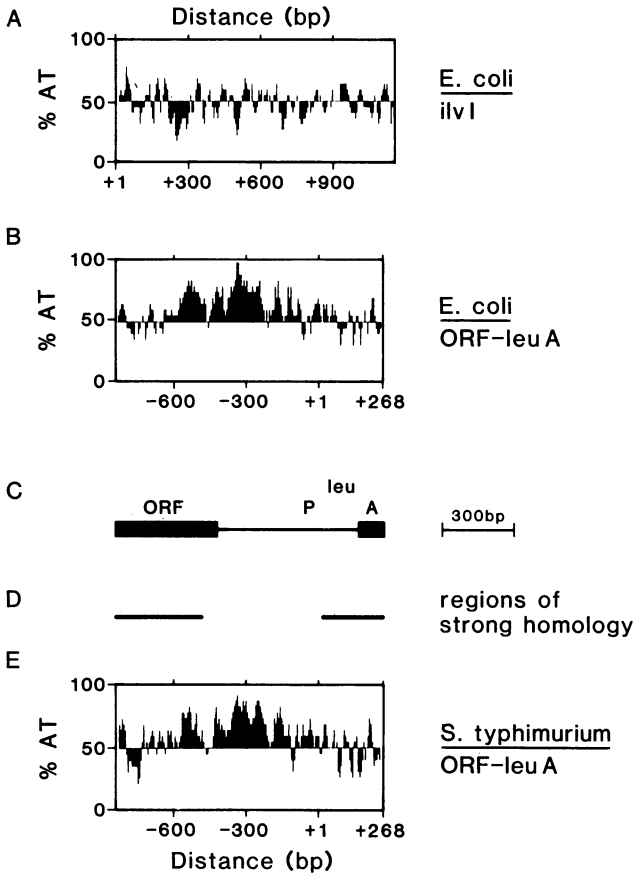

FIG. 2. A+T content of sequences in the *ilvI-leu* region. For A, B, and E, vertical lines represent the percentage of A+T in a 20-nucleotide region centered at the position indicated on the abscissa. (A) *E. coli ilvI* DNA (24). +1 corresponds to the position of the initiation codon of *ilvI* (13). (B and E) Respectively, *E. coli* and *S. typhimurium leuPL* and part of *leuA* together with sequences upstream of *leu*. Numbering corresponds to that shown in Fig. 1 with positive numbers representing distance (in base pairs) downstream of the *leu* transcription start site. (C) Schematic representation of the region of *leuA-leuP* and upstream sequences. (D) Regions of strong homology (——) between *E. coli* and *S. typhimurium* (Fig. 3).

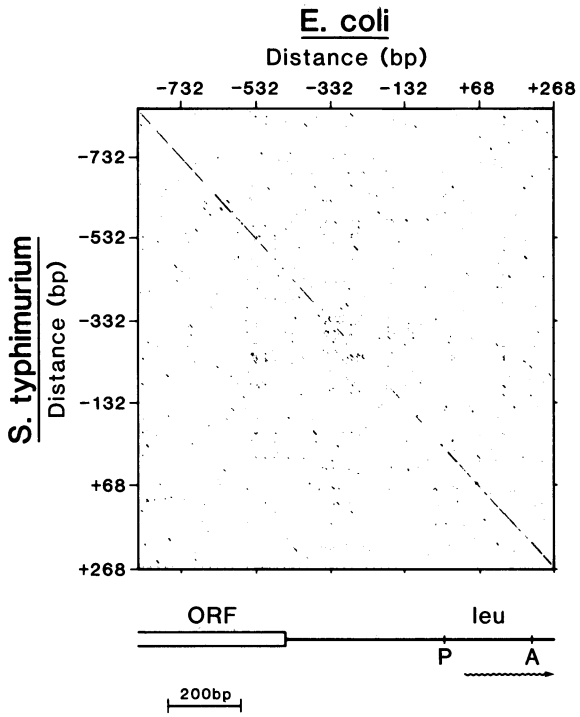

FIG. 3. Graphic matrix plot of nucleotide sequences within and upstream of the *E. coli leu* operon against the corresponding sequence in *S. typhimurium*. Dots represent instances in which 8 nucleotides out of 10 matched. The position of each dot corresponds to the center of the 10 nucleotides compared from each sequence. The numbering corresponds to that given in Fig. 1. Positive numbers indicate positions downstream of the *leu* transcription start site. The positions of *leu* and ORF sequences are indicated. The wavy arrow represents the direction of transcription of the *leu* operon.

substitution. It seems that A+T richness per se has been conserved in these organisms during evolution.

To determine whether the A+T-rich region plays a role in the expression of the adjacent *leu* operon, the following experiments were done. The *leu* promoter from plasmids pCV57 (region upstream from *leu* deleted) and plasmid pCV35 (upstream region present) were positioned upstream of *galk* on plasmid pK01 (Fig. 4). The level of galactokinase in strains containing these pK01 derivatives is a measure of the extent of transcription initiated in the direction of *galK* from the inserted promoter (16). pK01-derived plasmids carrying the *leu* or *lacUV5* (28) promoters give rise to 10 to 20 times more galactokinase activity than does plasmid pK01 itself (Table 1). The removal of sequences upstream of the *leu* promoter resulted in an apparent, approximately twofold decrease in *leu*-promoter strength. To determine if plasmid copy number was affecting the levels of galactokinase (1, 26), the copy number of pK01 derivatives was determined relative to plasmid pSE150. Plasmid pSE150, a low copy number plasmid derived from plasmid pDPT427 (23), was shown to be a useful internal standard for plasmid copy number determinations of *ilvIH* promoter-pK01 fusions (10). In the experiments reported here, the plasmid copy number of pK01 derivatives varied less than twofold among the strains examined.

The results described above demonstrate that optimal transcription from the *E. coli* promoter is affected only modestly by the deletion of upstream sequences. It is possible that the twofold decrease in promoter strength associated with the deletion is due to changes in the −40 to −50 region of the promoter (7, 11, 21). We rated the

FIG. 1. Nucleotide sequence of the region upstream of the *leuABCD* operons of *E. coli* (A) and *S. typhimurium* (B). Appropriate restriction fragments were isolated from plasmids pCV21 (8) (contains *S. typhimurium leuP-leuA* and upstream sequences), pCV35 (24) (contains *E. coli ilvIH-leuABCD*), and pCV58 and pCV60 (Bal 31 deletion derivatives of plasmid pCV35 carrying *leu* operon and upstream sequences. They were constructed as described in the legend to Fig. 4 for plasmid pCV57; Haughn and Calvo, unpublished data). Fragments were labeled with ^{32}P at 5′ or 3′ ends. The sequences shown were determined by the methods of Maxam and Gilbert (15) as modified by Smith and Calvo (22). The first nucleotide is the transcription start site of the *leu* operon. The −10 and −35 regions of the *leu* promoter are underscored by asterisks. The amino-terminal sequences of a putative polypeptide encoded by a large ORF on the *leu* noncoding strand of both organisms is given.

TABLE 1. Specific activity of galactokinase and the relative plasmid copy number of strains carrying pK01-derived plasmids

Strain[a]	Plasmid	Promoter upstream of *galK*	Sequence upstream of *leu*	Sp act of galactokinase[b]	Relative[c] plasmid copy number
CV781	pK01, pSE150	None		26	8.4
CV782	pK0110, pSE150	*lacUV5*		450	9.2
CV783	pCV61, pSE150	*leu*	Absent	250	8.5
CV785	pCV63, pSE150	*leu*	Present	530	7.0

[a] Plasmids were carried by host strain W3102 (*galK*) (2).
[b] Galactokinase activity was determined as described by Haughn et al. (10) and is expressed in nanomoles of galactose phosphate formed per minute per milligram of protein. Activities are not corrected for plasmid copy number.
[c] Plasmid copy number was determined as described by Haughn et al. (10). Relative plasmid copy number is the number of pK01-derived plasmid molecules per molecule of low-copy-number plasmid pSE150.

leu-promoter sequences on plasmids pCV63 (wild type) and pCV61 (−40 to −50 region replaced with *Eco*RI linker sequences) on the basis of their degree of similarity to a promoter consensus sequence (11) by the method of Mulligan et al. (18). The rating of the *leu* promoter on plasmid pCV61 (49.7) was lower than that of the wild-type promoter on plasmid pCV63 (51.5). This decrease in promoter rating predicts a 1.5-fold decrease in promoter strength (18), a value similar to the twofold decrease observed.

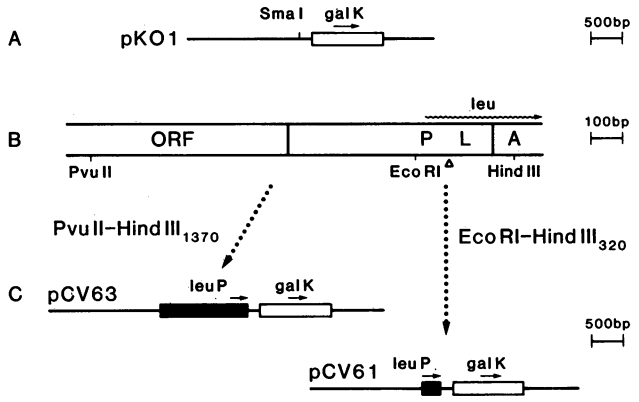

FIG. 4. The construction of plasmids pCV61 and pCV63. (A) Linear representation of plasmid pK01 showing positions of the unique *Sma*I site and *galK* gene (□). The arrow indicates the direction of transcription of *galK*. (B) Schematic representation of a region of the *E. coli* chromosome upstream of the *leu* operon. Plasmid pCV35 contains all of the region shown (25). To construct plasmid pCV57, containing a deletion of DNA upstream of *leu* to position −39, plasmid pCV35 was linearized with *Kpn*I, digested with nuclease Bal 31 and DNA fragments were cloned into the *Eco*RI site of plasmid pBR322 after ligation to an *Eco*RI dodecamer linker (GAGGAATTCCTC) and digestion with *Eco*RI. Plasmids able to transform strain SF8 (*hsdM hsdR leu thr thi str ligA recB recC*) to leucine prototrophy were characterized by restriction analysis of DNA prepared by the technique of Birnboim and Doly (3) and by DNA sequence analysis (22). The introduced *Eco*RI site is indicated by a triangle and the direction of transcription is indicated by a wavy line. (C) The *Pvu2-Hin*dIII fragment 1370 from plasmid pCV35 and the *Eco*RI-*Hin*dIII fragment 320 from plasmid pCV57 were isolated and their ends made blunt by treatment with avian myeloblastosis virus reverse transcriptase (a gift of J. Beard). Fragments were combined with *Sma*I-cut pK01 and treated with T_4 DNA ligase (New England Biolabs, Inc., Beverly, Mass.). The ligated DNA was digested with *Sma*I to linearize molecules of pK01 carrying no insert. Ampicillin-resistant colonies of strain W3102 (*galK*$^-$) were selected after transformation with ligated DNA. Plasmid DNA from selected colonies was isolated by the rapid technique of Birboim and Doly (3) and screened by restriction analysis. The solid rectangles represent the inserted sequences, and the short arrows indicate the direction of transcription from the *leu* promoter and of *galK*.

Similar studies on the in vivo effect of the removal of A+T-rich sequences upstream of the bacteriophage λ promoter P_L also demonstrate only small changes in promoter strength (12). We cannot exclude the possibility that deletion of A+T-rich upstream sequences would have more pronounced effects on transcription if these promoters were on the chromosome.

Assuming that the evolutionarily conserved A+T-rich region upstream of the *leu* operon has a function, what might that function be? If ORF is a gene, then it is transcribed in a direction opposite to that of *leu*, so that the A+T-rich region lies upstream of both operons. Conceivably, the A+T-rich regions plays some role in the expression of the ORF region. Alternatively, extensive A+T-rich regions may be positioned periodically on the chromosome as has been described for avian and mammalian genomes (17). The *E. coli* chromosome is known to be supercoiled and folded into domains (30). Localized denaturation of A+T-rich regions may stabilize folding or supercoiling by relieving torsion on the DNA helix. Yet another potential role for an A+T-rich region is that it may act as an entry site for proteins that interact with DNA. Proteins such as RNA polymerase are known to preferentially bind A+T-rich DNA (4, 27). It will be of interest to learn if other extensive A+T-rich regions exist in the chromosome of enteric bacteria.

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