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Nucleotide sequence of the alpha ribosomal protein operon of *Escherichia coli*

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ABSTRACT

In Escherichia coli some 19 transcription units encoding the 52 ribosomal proteins are scattered throughout the genome. One of the units, the alpha operon, encodes genes for the ribosomal proteins S13, S11, S4 and L17 as well as the alpha subunit of RNA polymerase. We report here the complete 3.0 kb nucleotide sequence of the alpha operon. In addition, we have determined by S1 nuclease mapping the site of transcription termination in this operon.

INTRODUCTION

The Escherichia coli ribosome consists of 52 ribosomal proteins (r-proteins) and 3 rRNA species. The genes for the r-proteins are organized into about 19 transcription units mapping at different chromosomal locations (1). Twenty-seven of these r-protein genes are located in a region traditionally referred to as the str-spc region at 72 minutes of the E. coli genetic map (2,3). This region is organized into 4 transcription units, termed the str, S10, spc, and alpha "operons" (2-4). Recent experiments have shown that during exponential growth the spc and alpha operons are at least partially co-transcribed (5), suggesting that the alpha operon promoter previously identified (6-8) may not function in exponentially growing cells.

In addition to genes for r-proteins, both the spc and the alpha operons are endowed with information for proteins which are not believed to be directly involved in protein synthesis. The spc operon contains in addition to genes for 10 r-proteins a gene (prlA or secY) implicated in protein secretion (9,10). The alpha operon harbors the gene for the alpha subunit of RNA polymerase (11) as well as the genes for r-proteins S13, S11, S4 and L17. To the extent that the spc and alpha operons are cotranscribed, this region thus represents an 8.6 kb transcription unit encoding 16 proteins (and possibly an additional protein called X; refs. 5,7,12) involved in

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transcription, translation and protein secretion.

The nucleotide sequence of the entire spc operon and parts of the alpha operon have previously been published (5,7,13,14). We have now completed the nucleotide sequence of the alpha operon. We have also located the site of transcription termination just distal to the L17 gene, the last known gene of the alpha operon. Interestingly, the apparent termination signal shows extensive homology with the putative transcription termination signal of the beta-operon which encodes two other subunits of the RNA polymerase.

MATERIALS AND METHODS

Strains

Bacteriophages M13mp8, M13mp9, M13mp10, M13mp11, M13mp18 and M13mp19 (15-17) were grown in JM103 (18) as described in the M13 cloning manual published by Bethesda Research Laboratories, Inc. The strain JM103 (pNO2530) was used as a source of RNA for the S1 mapping experiments. The plasmid pNO2530 contains the alpha and L17 genes (Fig. 1) expressed from the lac promoter on this plasmid (D. Bedwell and M. Nomura, unpublished experiments).

DNA Sources

The ultimate sources of DNA used for sequencing were λfus3, λspc1 and λspc2 (2,50). The 2.5 kb EcoRI fragment obtained from λfus3 or λspc1 (3) as well as the 1.7 kb PstI fragment from λspc2 (7) were used to sequence the S13, S11 and S4 genes. The sequence of the distal half of the alpha gene and most of the L17 gene was obtained from a 0.9 kb EcoRI/PstI (14,19) fragment from λspc2Δ16 (2) or λspc1. A 3.2 kb PstI fragment from λspc2 or λfus3 (14,19) was used as the DNA source for sequencing the end of the L17 gene as well as the termination region. Various segments of the above DNA fragments were subcloned into M13mp8, M13mp9, M13mp10, M13mp11, M13mp18 or M13mp19, using the restriction enzymes indicated in Figure 1. Restriction enzymes and DNA ligase were purchased from New England Biolabs, Bethesda Research Laboratories, or Boehringer Mannheim, or were prepared by Eric Lifson in Rochester.

DNA Sequencing

Most of the DNA sequencing was done by the dideoxynucleotide chain termination method of Sanger et al. (20), in some cases as modified for [^{35}S]nucleotides by Biggin et al. (21). Klenow fragment of DNA polymerase I was obtained from New England Biolabs, Boehringer Mannheim or IBI Biochemicals. The M13 universal primer was obtained from P.L. Biochemicals.

Sequencing gels were prepared as described previously (5,22). The sequences of portions of the S11 and S4 genes and the terminator region were determined both by the method of Maxam and Gilbert (23) and the dideoxy method. The transcription termination region contains an inverted repeat which can form a hairpin structure (see below). This caused problems with premature termination in the primer extension reactions used for the dideoxy sequencing as well as with compression of bands in the sequencing gels. Some of these problems were alleviated by substituting dITP for dGTP in the primer extension reaction mixtures as suggested by Mills and Kramer (24). Furthermore we confirmed the sequence of the DNA containing the possible hairpin structure by dideoxy sequencing of the PstI-HpaII and RsaI-HpaII fragments positioned on either side of HpaII site in the loop at the top of the hairpin (position 8802). DNA sequence analysis was done with programs provided through the University of Wisconsin Genetics Computer Group, or with the microcomputer sequence analysis programs of Pustell (25,26).

S1 Nuclease Mapping

In the S1 mapping of the transcription termination site, the DNA fragment (see legend to Fig. 3) was 3' end labeled with Klenow fragment (27), incubated with RNA and then digested with S1 nuclease by the method of Berk and Sharp (28), essentially as described by Barry et al. (29). The products were run on a DNA sequencing gel next to the same radioactively labeled DNA fragment treated with the chemical reactions of Maxam and Gilbert (23). RNA for this experiment was prepared from a culture of JM103 (pNO2530) grown in LB medium supplemented with ampicillin at 50 ug/ml. The same results were obtained with RNA from cells grown in the presence or absence of 1.5 mM IPTG (D. Bedwell and M. Nomura, unpublished). RNA was purified by the hot phenol method (30). S1 nuclease was purchased from Bethesda Research Laboratories.

RESULTS AND DISCUSSION

DNA Sequence of the alpha Operon

We present here the completed DNA sequence of the alpha operon. Three segments of this operon have previously been reported (Fig. 1). These are: (a) the promoter region through the proximal 109 base pairs of the S13 gene (7), (b) the distal 180 base pairs of the S4 gene through the first 378 base pairs of the alpha gene (13) and (c) the distal part of the alpha gene and the entire L17 gene (but not the terminator region following the L17 gene; ref. 14). We now add to these published sequences new data that complete

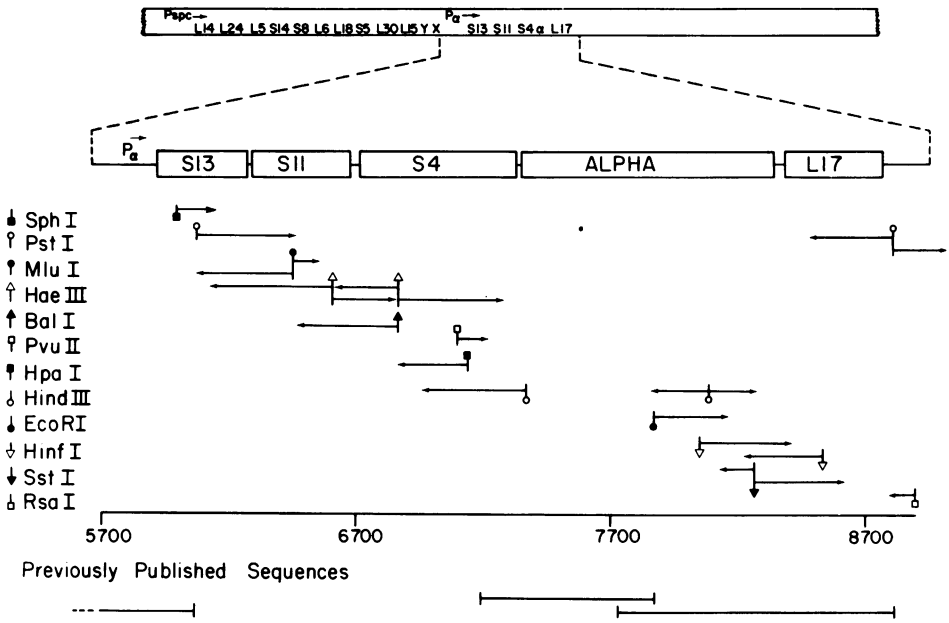
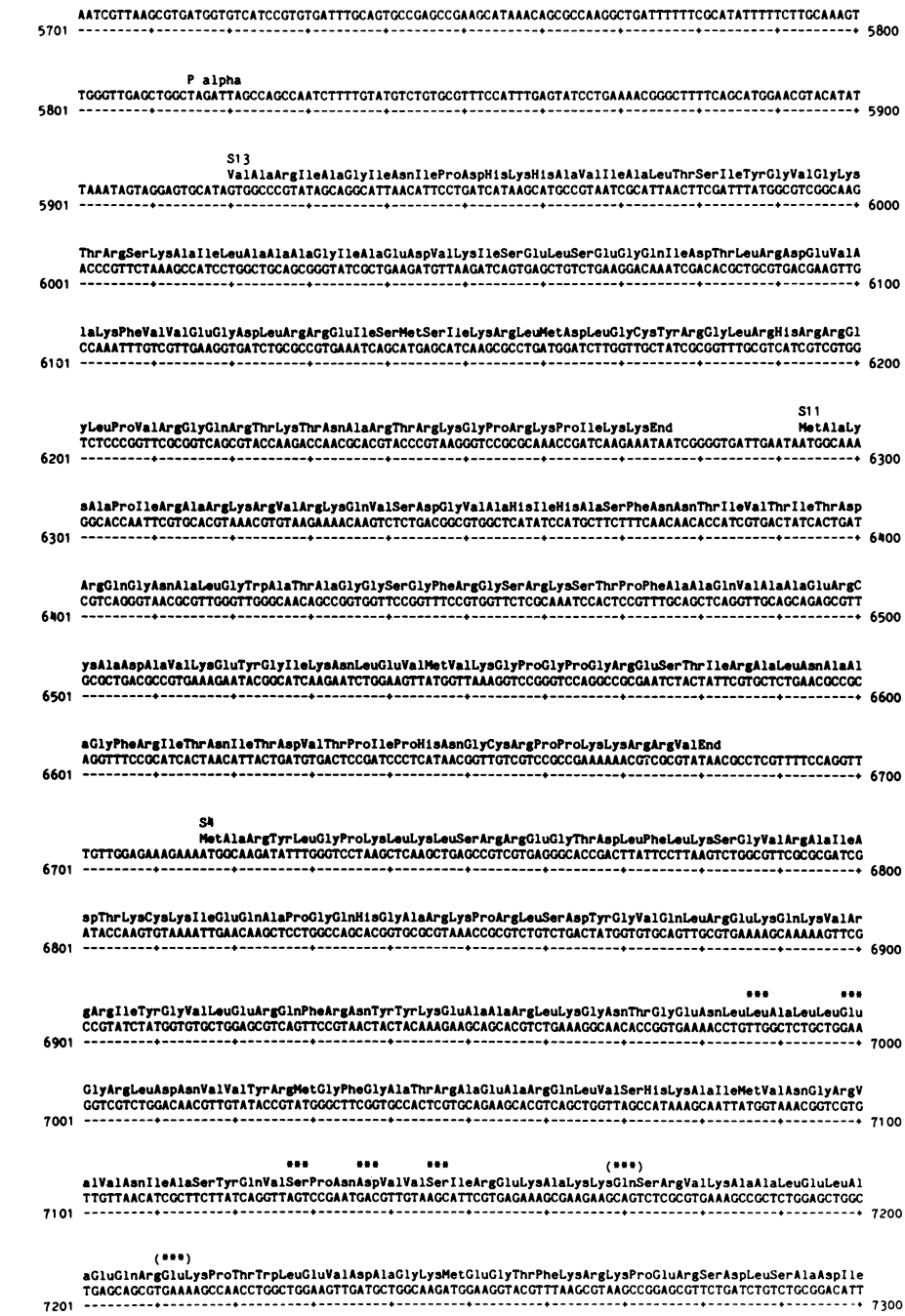
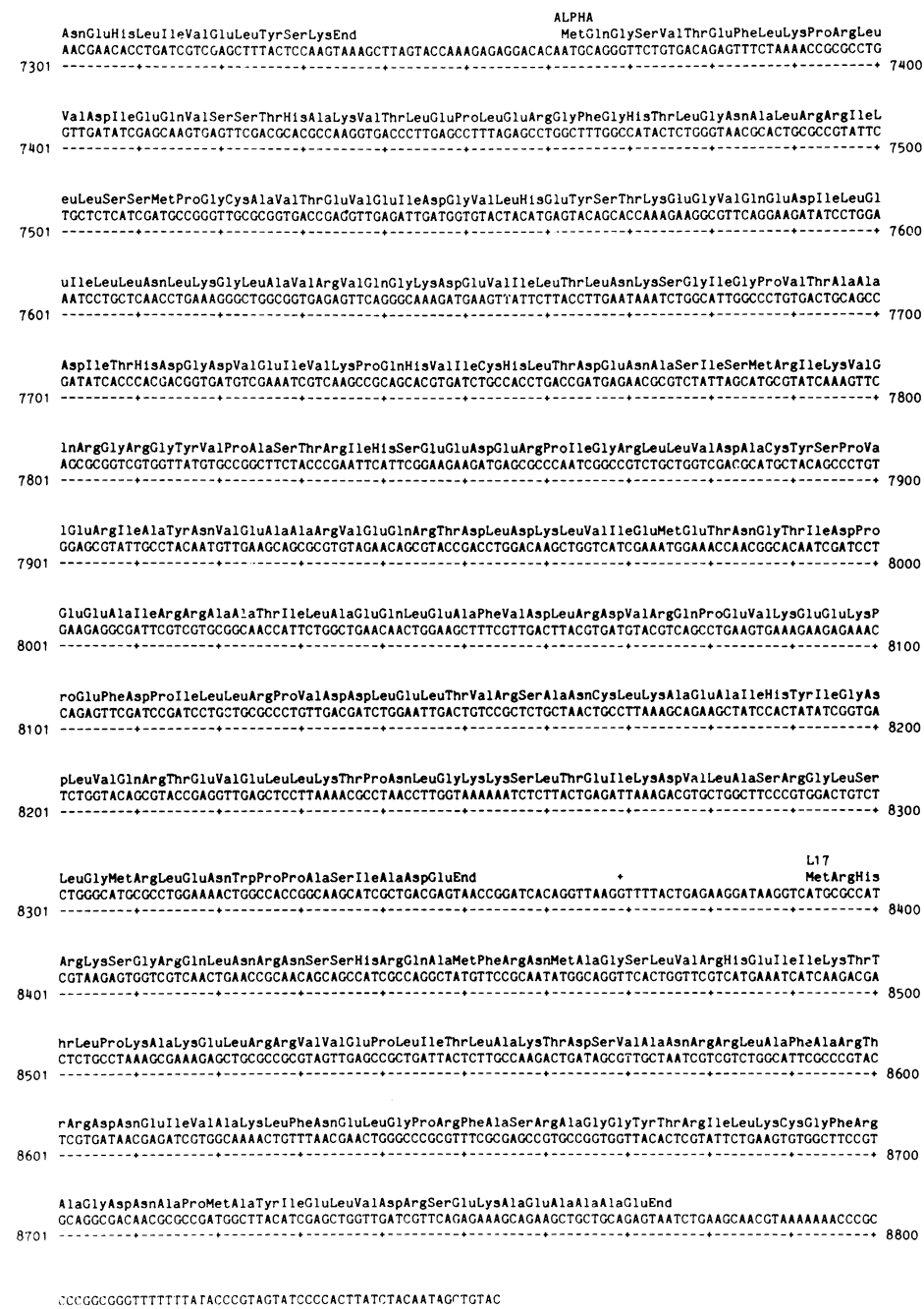


Fig. 1. Map of the spc and alpha operons and sequencing strategy for the alpha operon. The locations of the promoters and r-protein genes of the spc and alpha operons are shown in the upper bar. Sequence determination was done by the dideoxy method of Sanger (20). Portions of the S11 and S4 genes, as well as the terminator region, were also done by the method of Maxam and Gilbert (23). Only relevant restriction enzyme sites are indicated. Horizontal arrows indicate the direction and distance sequenced from a single restriction site. The scale at the bottom represents the numbering of the nucleotide bases used in Fig. 2 and is a continuation of the numbering of the spc operon sequence (5).

the sequence of the entire operon, including the transcription terminator region following the last gene of the operon (the L17 gene). Our sequence deviates at one point from that presented by Meek and Hayward (14). For this reason and for the convenience of the reader, we report the sequence of the entire alpha operon.

DNA derived from λspc1, λspc2 or λfus3 (2) was sequenced employing the strategy shown in Fig. 1 and in the Materials and Methods. The complete nucleotide sequence of the alpha operon is presented in Fig. 2. All of the new sequence data presented here were derived either from both DNA strands or from independent, overlapping clones of the same strand. In addition, most of the new sequences have been determined independently by both the laboratories contributing to this publication.




ALPHA
AsnGluHisLeuIleValGluLeuTyrSerLysEnd
MetGlnGlySerValThrGluPheLeuLysProArgLeu
AACGAACACCTGATCGTCGAGCTTTACTCCAAGTAAAGCTTAGTACCAAAGAGAGGACACAATGCAGGGTTCTGTGACAGAGTTTCTAAAACCGCGCCTG
7301
7400
ValAspIleGluGlnValSerSerThrHisAlaLysValThrLeuGluProLeuGluArgGlyPheGlyHisThrLeuGlyAsnAlaLeuArgArgIleL
GTTGATATCGAGCAAGTGAGTTCGACGCACGCCAAGGTGACCCTTGAGCCTTTAGAGCCTGGCTTTGGCCATACTCTGGGTAACGCACTGCGCCGTATTC
7401
7500
euLeuSerSerMetProGlyCysAlaValThrGluValGluIleAspGlyValLeuHisGluTyrSerThrLysGluGlyValGlnGluAspIleLeuGl
TGCTCTCATCGATGCCGGGTTGCGCGGTGACCGACGTTGAGATTGATGGTGTACTACATGAGTACAGCACCAAAGAAGGCGTTCAGGAAGATATCCTGGA
7501
7600
uIleLeuLeuAsnLeuLysGlyLeuAlaValArgValGlnGlyLysAspGluValIleLeuThrLeuAsnLysSerGlyIleGlyProValThrAlaAla
AATCCTGCTCAACCTGAAAGGGCTGGCGGTGAGAGTTCAGGGCAAAGATGAAGTTATTCTTACCTTGAATAAATCTGGCATTGGCCCTGTGACTGCAGCC
7601
7700
AspIleThrHisAspGlyAspValGluIleValLysProGlnHisValIleCysHisLeuThrAspGluAsnAlaSerIleSerMetArgIleLysValG
GATATCACCCACGACGGTGATGTCGAAATCGTCAAGCCGCAGCACGTGATCTGCCACCTGACCGATGAGAACGCGTCTATTAGCATGCGTATCAAAGTTC
7701
7800
lnArgGlyArgGlyTyrValProAlaSerThrArgIleHisSerGluGluAspGluArgProIleGlyArgLeuLeuValAspAlaCysTyrSerProVa
ACCGCGGTCGTGGTTATGTGCCGGCTTCTACCCGAATTCATTCGGAAGAAGATGAGCGCCCAATCGGCCGTCTGCTGGTCGACGCATGCTACAGCCCTGT
7801
7900
lGluArgIleAlaTyrAsnValGluAlaAlaArgValGluGlnArgThrAspLeuAspLysLeuValIleGluMetGluThrAsnGlyThrIleAspPro
GGAGCGTATTGCCTACAATGTTGAAGCAGCGCGTGTAGAACAGCGTACCGACCTGGACAAGCTGGTCATCGAAATGGAAACCAACGGCACAATCGATCCT
7901
8000
GluGluAlaIleArgArgAlaAlaThrIleLeuAlaGluGlnLeuGluAlaPheValAspLeuArgAspValArgGlnProGluValLysGluGluLysP
GAAGAGGCGATTCGTCGTGCGGCAACCATTCTGGCTGAACAACTGGAAGCTTTCGTTGACTTACGTGATGTACGTCAGCCTGAAGTGAAAGAAGAGAAAC
8001
8100
roGluPheAspProIleLeuLeuArgProValAspAspLeuGluLeuThrValArgSerAlaAsnCysLeuLysAlaGluAlaIleHisTyrIleGlyAs
CAGAGTTCGATCCGATCCTGCTGCGCCCTGTTGACGATCTGGAATTGACTGTCCGCTCTGCTAACTGCCTTAAAGCAGAAGCTATCCACTATATCGGTGA
8101
8200
pLeuValGlnArgThrGluValGluLeuLysThrProAsnLeuGlyLysSerLeuThrGluIleLysAspValLeuAlaSerArgGlyLeuSer
TCTGGTACAGCGTACCGAGGTTGAGCTCCTTAAAACGCCTAACCTTGGTAAAAAATCTCTTACTGAGATTAAAGACGTGCTGGCTTCCCGTGGACTGTCT
8201
8300
L17
LeuGlyMetArgLeuGluAsnTrpProProAlaSerIleAlaAspGluEnd
MetArgHis
CTGGGCATGCGCCTGGAAAACTGGCCACCGGCAAGCATCGCTGACGAGTAACCGGATCACAGGTTAAGGTTTTACTGAGAAGGATAAGGTCATGCGCCAT
8301
8400
ArgLysSerGlyArgGlnLeuAsnArgAsnSerSerHisArgGlnAlaMetPheArgAsnMetAlaGlySerLeuValArgHisGluIleIleLysThrT
CGTAAGAGTGGTCGTCAACTGAACCGCAACAGCAGCCATCGCCAGGCTATGTTCCGCAATATGGCAGGTTCACTGGTTCGTCATGAAATCATCAAGACGA
8401
8500
hrLeuProLysAlaLysGluLeuArgArgValValGluProLeuIleThrLeuAlaLysThrAspSerValAlaAsnArgArgLeuAlaPheAlaArgTh
CTCTGCCTAAAGCGAAAGAGCTGCGCCGCGTAGTTGAGCCGCTGATTACTCTTGCCAAGACTGATAGCGTTGCTAATCGTCGTCTGGCATTCGCCCGTAC
8501
8600
rArgAspAsnGluIleValAlaLysLeuPheAsnGluLeuGlyProArgPheAlaSerArgAlaGlyGlyTyrThrArgIleLeuLysCysGlyPheArg
TCGTGATAACGAGATCGTGGCAAAACTGTTTAACGAACTGGGCCCGCGTTTCGCGAGCCGTGCCGGTGGTTACACTCGTATTCTGAAGTGTGGCTTCCGT
8601
8700
AlaGlyAspAsnAlaProMetAlaTyrIleGluLeuValAspArgSerGluLysAlaGluAlaAlaAlaGluEnd
GCAGGCGACAACGCGCCGATGGCTTACATCGAGCTGGTTGATCGTTCAGAGAAAGCAGAAGCTGCTGCAGAGTAATCTGAAGCAACGTAAAAAAACCCGC
8701
8800
CCCGGCGGGTTTTTTTATACCCGTAGTATCCCCACTTATCTACAATAGCTGTAC
9801
8854

The nucleotide sequence confirms the gene organization of the alpha operon previously determined from biochemical and genetic experiments (2,7,11-13). In addition, it confirms and refines the published amino acid sequences for the five proteins encoded by the operon. The DNA sequence of the S13, S11, alpha and L17 genes show complete agreement with the published amino acid sequences of the protein products of these genes (31-34). However, we have found several discrepancies between the published amino acid sequence of the S4 protein (35) and the amino acid sequence deduced from the nucleotide sequence of the gene for this protein. Thus our DNA sequence predicts (a) an additional leu residue between amino acids 89 and 90; (b) glu at amino acid 93 instead of gln; (c) ser at amino acid 136 instead of asp; (d) asp at amino acid 139 instead of ser; (e) an additional ser residue between amino acids 141 and 142; (f) gln at amino acid 149 instead of glu; and (g) glu at amino acid 163 instead of gln (Fig. 2). The latter two discrepancies were noted previously (13).

We also found one discrepancy with the DNA sequence of the alpha/L17 intergenic region published by Meek and Hayward (14). They reported a C between the two G's at positions 8368 and 8369 of our sequence. Our results indicate that this C is not present. Each of the corrections mentioned above have been confirmed by sequencing both DNA strands as well as by independent experiments in Madison and Rochester.

The frequency of codon usage in the five genes of the alpha operon is shown in Table 1. Both the r-protein genes and the alpha gene show the highly non-random codon usage that has previously been observed for r-protein genes and other highly expressed genes (5,22,36). The preferentially used codons correspond to the most abundant tRNA species. The possible significance of this observation has previously been discussed (36-40).

Identification of the 3' End of the alpha Operon Transcript

The precise location of transcription termination at the end of the

Fig. 2. DNA sequence of the alpha operon. The numbering system is a continuation of that used for the DNA sequence of the spc operon (5). Parts of this sequence have been published previously (7,13,14). Transcripts initiated at the alpha promoter start at position 5806 (7). The differences between the amino acid sequence inferred from our DNA sequence and the amino acid sequence published for r-protein S4 (35) have been indicated with asterisks (***) above the corrected sequence. The differences indicated by asterisks in parentheses were previously noted (13). We also note one discrepancy between our DNA sequence and the one previously published (14) in the intergenic region between the alpha and L17 genes [indicated by a +] at position 8369. See text for further details.

Table 1. Codon Usage for the Alpha Subunit of RNA Polymerase and the Ribosomal Protein Genes of the Alpha Operon

Codons	r-Proteins	Alpha	Codons	r-Proteins	Alpha
Gly GGG	1	1	Trp TGG	2	1
Gly GGA	1	1	End TGA	0	0
Gly GGT	30	9	Cys TGT	3	0
Gly GGC	15	9	Cys TGC	2	4
Glu GAG	15	17	End TAG	0	0
Glu GAA	21	19	End TAA	4	1
Asp GAT	12	13	Tyr TAT	6	2
Asp GAC	10	8	Tyr TAC	7	3
Val GTG	8	11	Leu TTG	5	2
Val GTA	7	4	Leu TTA	2	2
Val GTT	20	10	Phe TTT	4	2
Val GTC	4	5	Phe TTC	10	2
Ala GCG	9	6	Ser TCG	1	3
Ala GCA	24	7	Ser TCA	2	1
Ala GCT	18	7	Ser TCT	12	7
Ala GCC	12	3	Ser TCC	3	1
Arg AGG	0	0	Arg CGG	0	0
Arg AGA	2	1	Arg CGA	0	1
Ser AGT	3	1	Arg CGT	49	14
Ser AGC	9	4	Arg CGC	18	7
Lys AAG	25	3	Gln CAG	11	8
Lys AAA	23	13	Gln CAA	5	2
Asn AAT	4	2	His CAT	10	3
Asn AAC	20	6	His CAC	2	5
Met ATG	9	4	Leu CTG	31	23
Ile ATA	1	0	Leu CTA	0	2
Ile ATT	1	10	Leu CTT	4	6
Ile ATC	22	14	Leu CTC	2	3
Thr ACG	3	3	Pro CCG	14	6
Thr ACA	1	2	Pro CCA	3	3
Thr ACT	14	4	Pro CCT	5	7
Thr ACC	9	11	Pro CCC	0	0

Codons listed do not include initiation codons.

alpha operon was determined by S1 nuclease mapping (29,41). A 139 base pair HinPI/RsaI DNA fragment labeled at the 3' end of the HinPI site (Fig. 3B) was hybridized with total cellular RNA made from cells carrying the P_{lac}-alpha-L17 plasmid pNO2530 (see Materials and Methods). We used RNA from both uninduced and isopropylthiogalactoside induced cells. In either case the 3'-ends of the alpha operon transcripts map in the run of U's of a

structure typical of a Rho-independent terminator (42). The results from the experiment with RNA from the uninduced cells is shown in Fig. 3A. Since no full-length hybridization probe was protected by RNA against S1 nuclease degradation, it is likely that essentially all transcription terminates at the indicated site. Note that the same termination sites were observed whether or not the plasmid borne P_{lac}-alpha-L17 operon was induced. Thus transcripts initiated both at the normal chromosomal promoter and at the lac promoter terminate at the same positions.

Interestingly, the terminator structure at the end of the alpha operon is preceded by another possible stem-loop structure (Fig. 3C) in a configuration very similar to that proposed for the end of the transcripts of the operon coding for the beta and beta' subunits of RNA polymerase (43; Fig. 3D). The similarity includes the identical sequence GAGUAAUC, with the UAA stop codon (for the last genes of the operons) located in the loop of the stem-loop structure preceding the terminator structures. In addition, the terminator stem-loop structures themselves are very similar with 10 identical bases pairs in the stems and loops of 4 bases. Transcripts from the operon encoding the sigma subunit of RNA polymerase also terminate at a Rho-independent terminator which is preceded by another stem-loop structure (41). However, the tandem stem-loop structures of the sigma operon differ both in structure and spacing from the corresponding structures of the alpha and beta operons. We do not know the significance of the very similar tandem stem-loop structures found in the alpha and the beta operons. In the case of the beta operon, it was previously suggested that the stem-loop structure may help to prevent degradation of mRNA by 3' exonucleases (43; see also 44). This suggestion may also apply to the alpha operon transcript. However, as mentioned above, practically all of the alpha operon mRNA terminates at the consecutive U's at the distal side of the second stem-loop structure. No transcripts of shorter length can be detected, making it unclear why the first stem-loop should be involved in protection against exonucleases.

Regulation of Protein Synthesis

Although the gene for the alpha subunit of RNA polymerase is cotranscribed with r-protein genes, the regulation of alpha synthesis is different from that of r-protein synthesis under some conditions. For example, r-protein synthesis is subject to stringent control during amino acid limitation, whereas the synthesis of alpha, like the synthesis of the other RNA polymerase subunits beta, beta' and sigma, is not subject to this

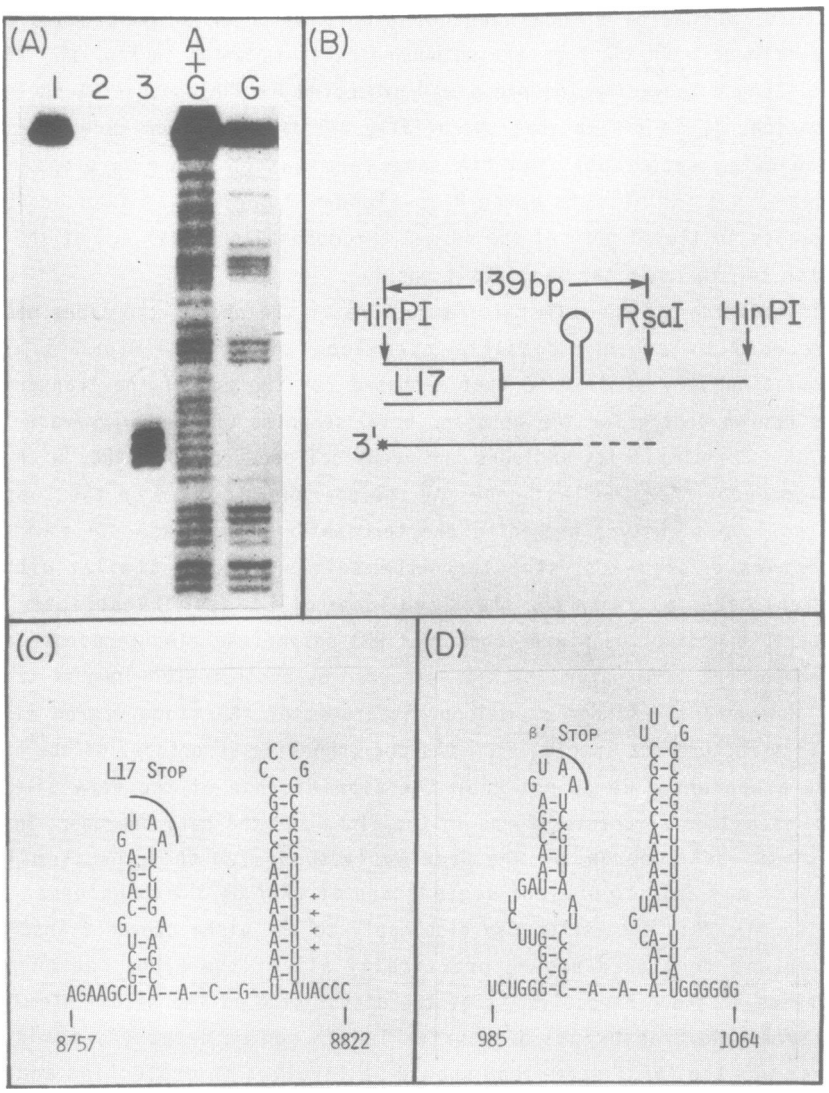


Fig. 3. S1 nuclease mapping of the transcription termination region of the alpha operon. The probe used is shown in (B). A 192 base pair HinPI fragment was 3' end labeled using [^{32}P]dGTP and Klenow fragment of DNA polymerase I. It was then cut with RsaI, and the purified 139 base pair HinPI/RsaI fragment was used as a hybridization probe. The results from such an experiment are shown in (A): Lane 1 shows the probe alone; lane 2 shows the probe hybridized to yeast RNA; lane 3 shows the probe hybridized to total RNA from E. coli JM103 (pNO2530) grown in LB without IPTG (essentially the same result was obtained with RNA from the same strain grown in LB with IPTG). The next two lanes show the same probe treated by the G and G+A sequence reactions of Maxam and Gilbert (23). Our

interpretation of these results are shown in (C), with arrows designating the termination points of the alpha operon mRNA. (C) also shows a probable secondary structure of the terminator region. The ΔG values calculated according to the revised Tinoco rules (49) for the proximal and distal stem-loop structures are -9.3 kcal/mol and -23.5 kcal/mol, respectively. (D) shows a similar secondary structure previously proposed for the end of the operon encoding the beta and beta' subunits of RNA polymerase (43). The calculated ΔG values for the proximal and distal stem-loop structures are -14.2 kcal/mol and -17.4 kcal/mol, respectively.

control (45,46; D. Bedwell and M. Nomura, unpublished). In the case of the beta and beta' genes, which are also cotranscribed with r-protein genes, differential regulation could be explained by attenuation in the region between the beta gene and the preceding r-protein genes (29,47). In the case of the gene for the sigma subunit, the presence of both an attenuator and a secondary promoter have been demonstrated and could account, at least in part, for the differences in regulation of the sigma gene and the upstream S21 gene (41).

The beta, beta' and sigma genes are located distal to the r-protein genes in their respective transcription units. The alpha gene is unique in the sense that it is located between r-protein genes. Therefore it is not clear how this gene could be regulated differently from the flanking r-protein genes. We have examined the nucleotide sequence for possible clues to this differential regulation but failed to find any sequence which might act as an attenuator or a secondary promoter. We have also performed S1 nuclease mapping with probes covering the region between 6028 (in the S13 gene) and the termination site to see if we could detect any transcripts which could reflect initiation or RNA processing within the alpha operon. No such transcripts were identified using RNA from either exponentially growing cells or from cells harvested during amino acid starvation conditions where differential regulation of alpha and r-protein synthesis was observed (D. Bedwell and M. Nomura, unpublished observations). These results therefore suggest that the differential regulation of alpha and r-protein synthesis during amino acid limitation may take place at the level of translation.

The autogenous regulation of the alpha operon also results in a differential effect on the synthesis of the r-proteins and alpha. Ribosomal protein S4, the product of the third gene of the alpha operon, inhibits the translation of the three r-protein cistrons on the proximal side as well as the L17 cistron on the distal side of the alpha gene (48). However, alpha synthesis is only partially affected by S4 (1,51). Inspection of the DNA

sequence of the alpha operon offers no obvious clues to the differential regulation of alpha and r-protein synthesis. Hopefully, the DNA sequence of the entire alpha operon presented in this paper will be useful for future studies of these and other questions related to the regulation of the genes in this operon.

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