JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5601–5606
0021-9193/88/125601-06$02.00/0

Vol. 170, No. 12

Highly Repetitive $tRNA^{Pro}$-$tRNA^{His}$ Gene Cluster from *Photobacterium phosphoreum*

SYLVIE GIROUX, JULIE BEAUDET, AND ROBERT CEDERGREN*

Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Received 6 July 1988/Accepted 19 September 1988

A DNA fragment comprising the four tRNA gene sequences of the *Escherichia coli argT* locus hybridized with two *Sau*3A-generated DNA fragments from the vibrio *Photobacterium phosphoreum* (ATCC 11040). Detailed sequence analysis of the longer fragment shows the following gene organization: 5′-promoter-$tRNA^{Pro}$-$tRNA^{Pro}$-$tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$-$tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$-five pseudogenes derived from the upstream $tRNA^{Pro}$ interspersed by putative Rho-independent terminators. This sequence demonstrates the presence of highly repetitive, tandem tRNA genes in a bacterial genome. Furthermore, a stretch of 304 nucleotides from this cluster was found virtually unchanged in the other (shorter) fragment which was previously sequenced. The two clusters together contain eight $tRNA^{Pro}$ pseudogenes and eight fully intact $tRNA^{Pro}$ genes, an unusually high number for a single eubacterial isoacceptor tRNA. These results show that the organization of some tRNA operons is highly variable in eubacteria.

Modern methods for the determination of evolutionary relationships among organisms have relied almost exclusively on the comparison of gene sequences (for examples, see references 9 and 25). Although gene sequence is a reliable evolutionary indicator, we have become increasingly interested in gene arrangement as a manifestation of ancestral relationships. This concern has been fueled to a large extent by increasing evidence for a certain fluidity in genome structure due to dynamic processes such as transposition, gene conversion, and various recombinational events (2, 22). To gather information on the importance of genome rearrangement as an evolutionary force, we have initiated a systematic study of tRNA gene organization among eubacteria. Owing to its essential role in translation as well as other functions and its putative early origin (15), tRNA gene arrangement and/or rearrangements may provide new and unique clues to the processes involved in genomic and organismal evolution.

Information concerning tRNA gene organization in various organisms has been accumulating; however, extensive knowledge of eubacterial organization is confined to *Escherichia coli* (10) and *Bacillus subtilis* (24). Surprisingly, despite the high similarity between sequences of corresponding tRNAs (tRNAs having the same anticodon sequence or isocoding tRNAs) in the two bacteria, the arrangement of tRNA genes differs considerably. With the exception of the tRNA genes found in the rRNA operon, tRNA genes in *B. subtilis* are found exclusively in three clusters containing 6, 16, and 21 tRNA genes (24). In *E. coli*, tRNA genes are much more highly dispersed; the largest tRNA operon is composed of only seven tRNA genes. Therefore, aside from $tRNA^{Ile}$ and $tRNA^{Ala}$ contained in the two rRNA operons in *B. subtilis* and the three rRNA operons of *E. coli*, no other tRNA gene clusters in the two bacteria are similar enough to show any common evolutionary heritage. It would seem that gene arrangement rather than gene sequence is more indicative of the classification of the two bacteria in relatively distant branches of the eubacterial kingdom: the gram-positive bacilli and the gram-negative enterobacteria.

* Corresponding author.

The study that we report here involved the use of the *argT* operon of *E. coli* as a probe of evolutionary relatedness (11). This operon is characterized by a simple organization of four tRNAs (5′-promotor-$tRNA^{Arg}$-$tRNA^{His}$-$tRNA^{Leu}$-$tRNA^{Pro}$-3′). Furthermore, the sequence of this operon was already known in *Salmonella typhimurium*, in which no rearrangements with respect to the *E. coli* structure were observed (3). In the first series of experiments, our analysis was confined to the purple bacteria and relatives group as defined by Woese and co-workers (26). We report here the characterization and the sequence of a tRNA cluster from *Photobacterium phosphoreum*, a phosphorescent marine bacterium of the family *Vibrionaceae*, which hybridized with the *E. coli* probe.

MATERIALS AND METHODS

Bacterial strains and vectors. The bacterial strains and vectors used in this study are listed in Table 1. All strains were grown in LB (10 g of tryptone [Difco Laboratories, Detroit, Mich.], 5 g of yeast extract, 5 g of NaCl per liter) except for *Vibrio harveyi* and *Photobacterium phosphoreum*, which were grown in a high-salt medium as described previously (S. Giroux and R. Cedergren, Proc. Natl. Acad. Sci. USA, in press). *V. harveyi* was grown at 20°C, *Photobacterium phosphoreum* was grown at 18°C, *Aeromonas hydrophila* was grown at 28°C, *Pseudomonas fluorescens* was grown at 26°C, and all other strains were grown at 37°C. DNA was extracted by the procedure described by Silhavy et al. (23).

Preparation of the *E. coli* probe and hybridization. Plasmid pLC25-25, a gift of M. J. Fournier, was digested with *Bam*HI and *Eco*RI under standard conditions. The digestion generated four fragments of 9, 7, 2, and 0.2 kilobases (kb), which were incubated with pBR327 digested with the same enzymes. The mixture was ligated and used to transform *E. coli* HB101 (*recA*). The clones were screened by digestion of plasmid DNA with restriction enzymes. The clone containing *argT* on a 1,935-base-pair (bp) *Eco*RI-*Bam*HI fragment was named pBE1935. To make a radioactive probe at *argT*, pBE1935 was digested with *Mlu*1 to give *argT* on a 0.8-kb fragment of DNA. A preparative *Mlu*1 digest of 20 μg of

TABLE 1. Bacteria and vectors used in this study

Strain or vector	Utilization	Source or reference
Escherichia coli MG1665	Prototroph (source of chromosomal DNA)	CSH 1987[a]
KK22186	F′ *traD36 proAB lacI*^{q} Δ(*lacZ*)M15 Δ(*lac pro*) *thi strA supE endA sbcB* r$^-$ m$^+$ (to propagate M13)	CSH 1987
HB101	RecA$^-$ (for transformation)	5
Proteus vulgaris	DNA source	UM[b]
Pseudomonas fluorescens	DNA source	UM
Aeromonas hydrophila	DNA source	UM
Photobacterium phosphoreum	DNA source	ATCC 11040
Vibrio harveyi	DNA source	ATCC 14126
pBR327	Cloning vector	1
M13mp19	Sequencing vector	16

[a] CSH, Cold Spring Harbor Laboratory.
[b] UM, Département de Microbiologie, Université de Montréal.

pBE1935 was electrophoresed on a 1% agarose gel, the 800-bp fragment was excised and electroeluted from the gel by using an electroeluter (model UEA; International Biotechnologies, Inc., New Haven, Conn.). The 800-bp fragment was labeled by using the oligolabeling kit from Pharmacia (Uppsala, Sweden) and [α-^{32}P]dCTP.

For hybridizations, 1 μg of genomic DNA from each bacterial strain was digested with *Eco*RI and *Bam*HI, the samples were electrophoresed on a 0.8% agarose gel along with a 1-kb fragment ladder (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The gel was transferred to Hybond-N (Amersham Corp., Arlington Heights, Ill.) by the alkaline procedure and hybridized with labeled *argT* according to the protocol furnished by the company.

Cloning procedure. A preparative *Hin*dIII digest of 20 μg of genomic DNA was electrophoresed through a 0.7% low-melting-point agarose gel. The region of the gel corresponding to 5 kb was excised and melted, and the DNA was purified by passage through Elutip-D (Schleicher & Schuell, Inc., Keene, N.H.). The DNA was recombined with *Hin*dIII-restricted pBR327 which had been dephosphorylated previously. The ligation mixture served to transform *E. coli* HB101; colonies were screened by colony hybridization by using the *Sau*3A insert from pPPS70 as a probe (Giroux and Cedergren, in press).

Sequencing procedure. Fragments for sequencing were purified in an agarose gel as described above and recombined with *Sma*I-restricted M13mp19 and *Bam*HI-restricted M13mp19. Both directions were isolated from *Bam*HI-M13 subclones by using synthetic probes complementary to the coding strand and anticoding strand of tRNA^{His}. A series of deletions was generated by using the procedure developed by Dale et al. (8). The sequences of both strands were determined by using the Sequenase DNA-sequencing kit from U.S. Biochemical Corp. (Cleveland, Ohio). To correctly align all the data, partial sequencing of the *Hae*III fragment was performed.

In vitro transcription assay. A purified restriction fragment containing the tRNA cluster was used as a single template for transcription. A 0.2-pmol sample of template was used per assay as described previously (19). Crude transcription mixtures were analyzed by electrophoresis on 5% polyacrylamide gels containing 8.0 M urea.

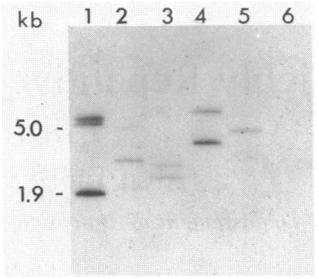

FIG. 1. Autoradiogram of [^{32}P]-labeled *argT* hybridized to restricted chromosomal DNA. Chromosomal DNA of different sources was digested simultaneously with *Eco*RI and *Bam*HI, fractionated by electrophoresis in a 0.8% agarose gel, transferred to Hybond-N, and hybridized with [^{32}P]-labeled *argT* as described in the text. The results shown are from restricted DNA from *E. coli* MG 1665 (lane 1), *Proteus vulgaris* (lane 2), *A. hydrophila* (lane 3), *Photobacterium phosphoreum* (lane 4), *V. harveyi* (lane 5), and *P. fluorescens* (lane 6).

RESULTS

***argT* from *E. coli*.** To obtain large amounts of the *argT* operon DNA, a 1.9-kb *Eco*RI-*Bam*HI restriction fragment from the low-copy-number plasmid pLC25-25 (ColE1) was subcloned into the multicopy plasmid pBR327. Transformants containing the operon insert had a slow-growth phenotype as evidenced by their small colony size. Also, as estimated from the quantity of plasmid DNA extracted from transformants, the plasmid copy number was much lower than expected for a normal 4.8-kb plasmid. Deletion of the part of the 1.9-kb DNA fragment not coding for tRNAs suggests that the slow-growth phenotype is dependent on the presence of the tRNA gene region. Possibly one of the tRNAs encoded in the high-copy-number plasmid is produced at an intracellular level which is deleterious to the growth of *E. coli*.

Since the presence of the plasmid-encoded tRNAs reduced cell growth, it seemed prudent to sequence a part of the tRNA gene insert to verify whether the gene order and/or gene sequences were modified in the positive transformants. The sequence of the region containing the four tRNA genes was found to be identical to the previously reported sequence (11). Nevertheless, we cannot exclude a possible mutation in the unsequenced upstream region which would affect tRNA expression as found in *S. typhimurium*. (4).

Southern hybridizations of genomic DNA. On the basis of the classification of the vibrio-enteric bacteria using 5S RNA sequences by MacDonell et al. (13), the following bacteria were selected: *Proteus vulgaris* (enteric), *A. hydrophila* (aeromonad), *Photobacterium phosphoreum* (vibrio), and *V. harveyi* (vibrio). These last two organisms represent two major *Vibrionaceae* genera. Additionally, the genomes of two pseudomonads, *P. fluorescens* and *Pseudomonas aeruginosa* (data not shown), which belong to a more distant branch in the gamma-3 subdivision, were analyzed.

The genome of each eubacterium was restricted and hybridized with ^{32}P-labeled *argT* DNA. Every member of the vibrio-aeromonad-enteric group gave a positive hybridization signal, but neither *P. aeruginosa* nor *P. fluorescens* did so (Fig. 1). On longer exposure times, some probe was seen to hybridize to an undefined distribution of high-

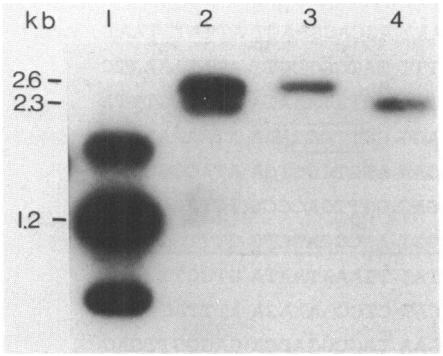

FIG. 2. Autoradiogram of [^{32}P]-labeled *argT* hybridized to restricted chromosomal DNA and plasmid DNA. Lanes: 1, 1 μg of chromosomal DNA from *E. coli* MG 1665 digested with *Sau*3A; 2, 1 μg of chromosomal DNA from *Photobacterium phosphoreum* digested with *Sau*3A; 3, 1 ng of pPPH12 DNA digested with *Sau*3A; 4, 1 ng of pPPS70 DNA digested with *Sau*3A.

molecular-weight DNA fragments (>15 kb) from the *Pseudomonas* species.

Cloning of *Photobacterium phosphoreum Sau*3A fragment. Digestion of *Photobacterium* DNA with *Sau*3A gave two genomic DNA fragments which hybridized with *argT* (Fig. 2). The band having the stronger signal is contained on the positive clone named pPPH12. The band having the weaker signal (the shorter fragment) cloned into pBR327 (called pPPS70) contains two $tRNA^{Pro}$ and one $tRNA^{His}$ gene (Giroux and Cedergren, in press). Since this fragment shows low organizational similarity to *argT*, we expected the second fragment, contained on pPPH12, to be more closely related. The complete nucleotide sequence is shown in Fig. 3. To certify that the sequences of the two cloned fragments have not been rearranged, we digested the plasmids containing each fragment as well as the genomic DNA with several restriction enzymes. Upon hybridization with the *argT* probe, fragments of identical size were obtained for the clones and genomic DNA (data not shown). Figure 4 is a schematic drawing of the possible secondary structure of a hypothetical RNA transcribed from this DNA. The DNA sequence revealed six identical $tRNA^{Pro}$ genes and two identical $tRNA^{His}$ genes. These genes were located by a visual search for the GTTC sequence of the tRNA T loop and confirmed by their ability to fold into the canonical cloverleaf structure of tRNA. Also, they contain key signature sequences which are specific for the two tRNA families (15). The tRNA gene sequences of pPPH12 are also identical to the $tRNA^{Pro}$ and $tRNA^{His}$ genes found in pPPS70 insert.

In addition, upstream of five of the $tRNA^{Pro}$ genes are structures 95 nucleotides long closely related to the 5′ half of the tRNA gene. The region of similarity with $tRNA^{Pro}$ spans the first 31 nucleotides 5′ to the anticodon loop and the 12 following nucleotides. Surprisingly, the anticodon loop has the same structure as that described for the $tRNA^{Pro}$ pseudogene of pPPS70, complete with the identical insertion sequence (CTGATGTTAGGAATAGC). The remaining portion of the segment shows no convincing similarity with the 3′ end of $tRNA^{Pro}$.

The entire cluster contains various duplicated sections. The largest hypothetical duplication involves 342 nucleotides from position 848 to position 1190 with the segment extending from position 1314 to position 1656. This segment includes the 95-nucleotide element discussed above, a $tRNA^{Pro}$ gene, a $tRNA^{His}$ gene, and the 5′ half of a $tRNA^{Pro}$ gene. Only four differences equivalent to 98% positional identity are found over these 342 bp. However, there is a stretch of 78 nucleotides between the two repetitive elements (from position 1238 to position 1315) that is unique in the cluster. This is in contrast to the other structures (the 95-nucleotide element, tRNA genes, and their spacers) which can be aligned with at least one other region of the cluster (Fig. 4) with a high level of positional identity (97 to 100%). This spacer sequence possesses only 61% positional identity with the two other spacers between the $tRNA^{Pro}$ gene and the 95-nucleotide element.

Promoter and terminator. A presumptive promoter can be identified 5′ to the first $tRNA^{Pro}$ gene. The sequences comprising the −35 box (TTGCTA at position 283) and the −10 box (TATTAT at position 305) are shown in Fig. 3; both are in good agreement with the consensus sequence for eubacterial promoters (for example, those of *B. subtilis* and *E. coli*). These elements are separated by 16 bp, a spacing that is considered optimal for most bacterial gene promoters sequenced thus far (14). The existence of a promoter on the *Sau*3A fragment is supported by the results from in vitro transcription with *E. coli* RNA polymerase (Fig. 5). The expected transcript of about 1,500 nucleotides from the promoter to the first Rho-independent terminator (see below) is present with other smaller transcripts.

In the 3′ region of the cluster, there are five potential hairpin loops terminated by a string of 8 thymidines which resemble Rho-independent terminators (18). This structure was also found in the shorter fragment (from pPPS70), where it was shown to serve as a terminator in an in vitro transcription assay (Giroux and Cedergren, in press). The results shown in Fig. 5 also support this interpretation.

Homology with pPPS70 insertion. Although neither of the two fragments from *Photobacterium phosphoreum* are similar to the *argT* locus except for the tRNA gene sequences themselves, the two fragments show remarkable similarity to each other. (i) The nature and the sequence of the tRNAs are the same (histidine and proline). (ii) The type of terminator, their sequences, and repetitive natures are virtually identical in both clusters, although the copy number is slightly different. (iii) Interspersed between these terminators are pseudogenes related to the $tRNA^{Pro}$ gene, with insertions in the anticodon loop and the T loop. (iv) The region of three tRNA genes in pPPS70 can be aligned with pPPH12 from position 941 to position 1244. The alignment without gaps covers 304 nucleotides with only three mismatches, representing more than 99% positional identity.

Differences between the two clusters include the presence of a 93-nucleotide element interspersed between terminators in pPPS70 and the 95-nucleotide element preceding the five $tRNA^{Pro}$ genes in pPPH12. In the case of pPPS70, most of the repetitive elements are clustered in the nontranscribed part of the operon (the pseudogene region), whereas in the cluster reported here, most repetitive elements include what seems to be functional tRNA genes.

DISCUSSION

The high copy number of $tRNA^{Pro}$ genes is completely unexpected, since in *E. coli* only two $tRNA^{Pro}$ genes coding for two different tRNA isoacceptors are presently known (10). Furthermore, the correlation that cellular tRNA levels are directly proportional to the use of their cognate codon (12) suggests that in *Photobacterium phosphoreum* either proline is used in protein sequences much more often than in other bacteria, such as *E. coli*, or $tRNA^{Pro}$ genes are subject

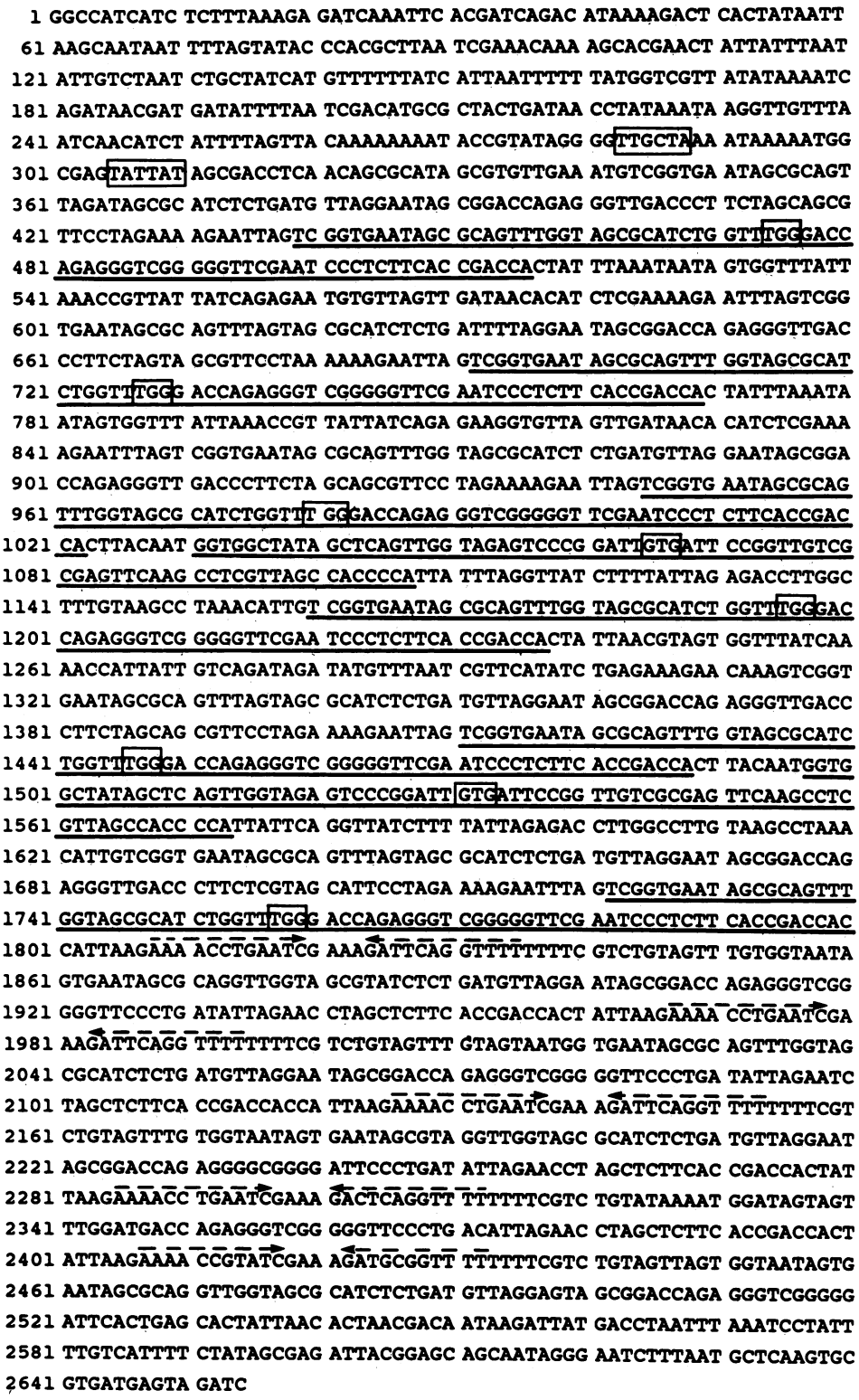

FIG. 3. DNA sequence of the 2,654-bp fragment isolated from *Photobacterium phosphoreum*. The sequence of the first 33 nucleotides was derived from the 1,600-bp *Hae*III fragment, and the other 2,621 bp was from the *Sau*3A fragment. The promoter elements are boxed, and each tRNA gene sequence is underlined, with its anticodon boxed. The terminator sequences are indicated with dashed arrows.

to differential regulation of expression. As for the first possibility, few data exist on the relative use of different amino acids in *Photobacterium phosphoreum*; however, proline is an amino acid that is not normally used to any great extent, and the relatively close relationship between *E. coli* and *Photobacterium phosphoreum* would argue against any major difference in proline use. In a compilation of codon use in sequenced vibrio genes involved in lumines-

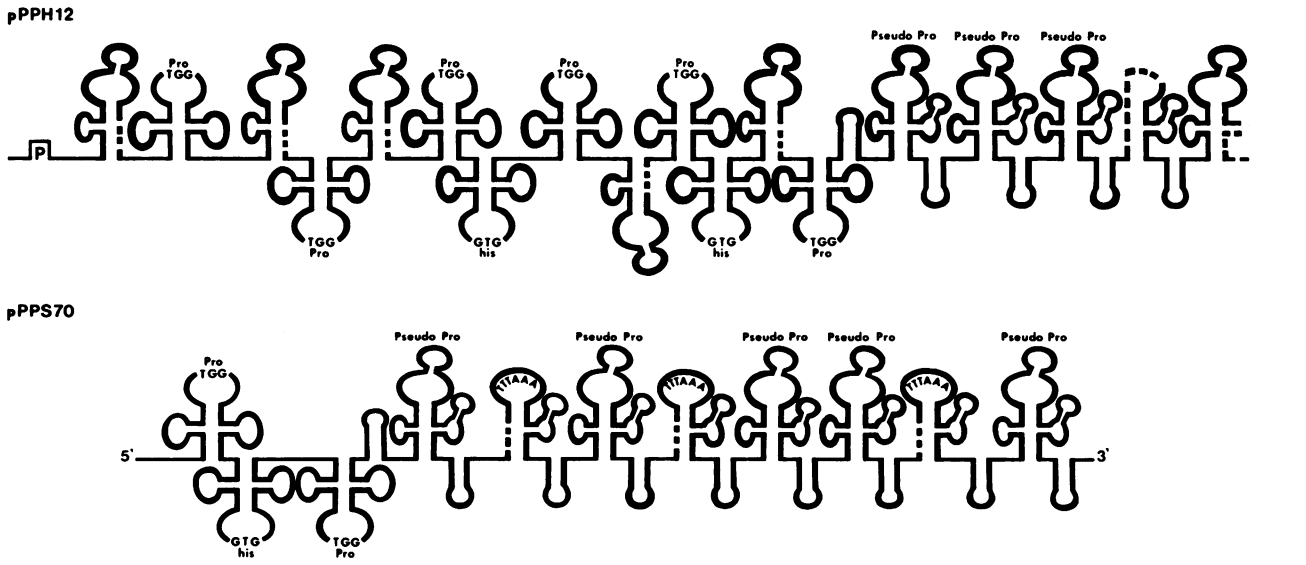

FIG. 4. Schematic drawing of the possible secondary structure of the sequenced fragment of *Photobacterium* DNA. For clarity, the inserts in the anticodon and T loops are indicated as hairpin structures; in reality these have no base-paired regions and increase the size of the loop. The dashed lines indicate that no similarity is found between this region and the 3′ half of $tRNA^{Pro}$.

cence kindly provided by E. Meighan, no evidence for increased proline use can be inferred. On the other hand, the presence of many gene copies of $tRNA^{Pro}$ could be useful in providing large amounts of pro-$tRNA^{Pro}$ during critical periods of the cell cycle or under certain conditions of environmental stress in *Photobacterium phosphoreum*. For a marine bacterium, such a stress could be osmotic, and it is well documented that proline is an osmoprotectant (7). Whether $tRNA^{Pro}$ has a role in this process is presently unknown.

The regulation of the intracellular level of $tRNA^{Pro}$ presupposes a strict control of its steady-state concentration either by modification of the half-life of tRNA degradation or by the transcription of normally inactive genes. Support for this latter suggestion is provided by the in vitro transcription studies presented here. Although a full-sized 1,500-nucleotide band is seen in Fig. 5, many shorter products are observed under conditions in which the *argT* operon of *E. coli* gives a full-length transcript. The nucleotide sequence of the cluster does contain possible terminator structures, i.e., weak hairpins, followed by A's and T's (18). Early termination of transcription is likely, since more recent work has shown the absence of cryptic promoters. Work is in progress to determine the nature of these transcriptional anomalies.

The origin of two tRNA clusters from *Photobacterium phosphoreum* has many interesting evolutionary aspects, but our desire to relate the arrangement of these tRNA genes is temporarily stymied, since both clusters are so different from the *E. coli* arrangement as to be uninterpretable within the framework of a common evolutionary ancestor. It is now incumbent upon us to more fully characterize the phylogenetic distribution of the repetitive genes and pseudogenes in order that a plausible scheme can be proposed. In ongoing experiments with a probe composed of the repetitive DNA from *Photobacterium phosphoreum*, preliminary information suggests that comparable structures are found in other vibrios, but not in either *A. hydrophila* or *E. coli*. The events leading to the repetitions would thus be relatively recent but not to the extent as to preclude the incorporation of some mutational drift.

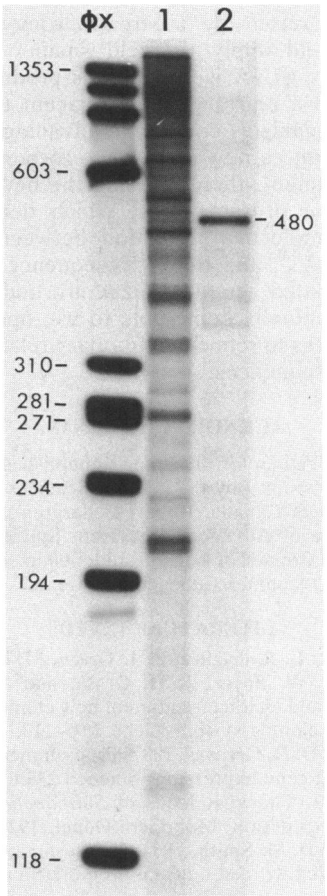

FIG. 5. In vitro transcription assay using purified restriction fragments as templates. Radioactive markers were prepared from φX174 DNA digested with *Hae*III and end labeled with [γ-^{32}P]ATP and T4 kinase. Lanes: 1, product from the transcription of the *Sau*3A restriction fragment from pPPH12; 2, product from the transcription of the *E. coli argT* which gave a major product of 480 nucleotides.

The observation that the two *Photobacterium phosphoreum* clusters have long tracks which are virtually identical is consistent with the view that gene conversion must have played some role in the similarity of these clusters (21). This fact must be integrated as well into an eventual model of the origin and history of the two clusters.

Finally, the present work relates to the overall question of tRNA gene arrangement. We have shown that even within relatively limited evolutionary space, major rearrangements of tRNA genes are possible. This is obviously not a general rule, since the tRNA genes found within the ribosomal operon are strictly conserved even in the chloroplast genome (17). Also, a tRNA operon from a mycoplasma is closely related to the corresponding operon in *B. subtilis* (20). Recently, Cantatore et al. (16) have proposed an explanation for tRNA gene rearrangements in mitochondria. This idea based on the initiation of mitochondrial DNA replication by tRNA may hold for mitochondria but cannot be applicable in a bacterial system.

Referring to the correlation of tRNA levels with the use of cognate codons especially in highly expressed proteins (12), it is tempting to speculate that tRNA gene rearrangements placing the gene in a new transcriptional environment could lead to pleiotropic effects. Protein genes containing relatively rare codons (decoded by rare tRNAs) could suddenly be expressed to higher levels than normal as the cognate tRNA level is raised by a strong promoter. The same mechanism would apply to yield smaller amounts of a protein, if a key tRNA isoacceptor is produced in smaller amounts due to a rearrangement adjacent to a weak promoter. Some organisms could take advantage of these new levels to move into a new niche or to respond better to the present environment, thereby fixing the new arrangement. At any rate the present work, which demonstrates the dichotomy of molecular evolution between gene (highly conserved $tRNA^{Pro}$ and $tRNA^{His}$ sequences) and genome evolution (unrelated gene organization), underlines the importance of eventually being able to use operons or entire genome structures to refine evolutionary relationships based solely on gene sequences.

ACKNOWLEDGMENTS

We thank E. Meighan for providing unpublished results.

This work is supported by the Natural Sciences and Engineering Research Council of Canada. Robert Cedergren is a fellow of the Canadian Institute of Advanced Research, Sylvie Giroux holds a Medical Research Council of Canada studentship, and Julie Beaudet received an FRSQ summer studentship.

LITERATURE CITED

1. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
2. **Borst, P., and D. R. Greaves.** 1987. Programmed gene rearrangements altering gene expression. Science **235**:658–667.
3. **Bossi, L.** 1983. The *his*R locus of *Salmonella*: nucleotide sequence and expression. Mol. Gen. Genet. **192**:163–170.
4. **Bossi, L., and D. M. Smith.** 1984. Conformational change in the DNA associated with an unusual promoter mutation in a tRNA operon of *Salmonella*. Cell **39**:643–652.
5. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
6. **Cantatore, P., M. N. Gadaleta, M. Roberti, C. Saccone, and A. C. Wilson.** 1987. Duplication and remolding of tRNA genes during the evolutionary rearrangement of mitochondrial genomes. Nature (London) **329**:853–855.
7. **Csonka, L. N.** 1988. Regulation of cytoplasmic proline levels in *Salmonella typhimurium*: effect of osmotic stress on synthesis, degradation, and cellular retention of proline. J. Bacteriol. **170**: 2374–2378.
8. **Dale, R. M., B. A. McClure, and J. P. Houchins.** 1985. Single-stranded cloning strategy. Plasmid **13**:31–40.
9. **Field, K. G., G. J. Olsen, D. J. Lane, S. J. Giovannoni, M. T. Ghiselin, E. C. Raff, N. L. Pace, and R. A. Raff.** 1988. Molecular phylogeny of the animal kingdom. Science **239**:748–753.
10. **Fournier, M. J., and H. Oseki.** 1985. Structure and organization of the transfer ribonucleic acid genes of *Escherichia coli* K-12. Microbiol. Rev. **49**:379–397.
11. **Hsu, L. M., H. J. Klee, J. Zagorski, and M. J. Fournier.** 1984. Structure of an *Escherichia coli* tRNA operon containing linked genes for arginine, histidine, leucine, and proline tRNAs. J. Bacteriol. **158**:934–942.
12. **Ikemura, T.** 1985. Codon usage and tRNA content in unicellular and multicellular organisms. Mol. Biol. Evol. **2**:13–34.
13. **MacDonell, M. T., D. G. Swartz, B. A. Ortiz-Coude, G. A. Last, and R. R. Colwell.** 1986. Ribosomal RNA phylogenies for the vibrio-enteric group of eubacteria. Microbiol. Sci. **3**:172–178.
14. **McClure, W. R.** 1985. Mechanism and control of transcription initiation in prokaryotes. Annu. Rev. Biochem. **54**:171–204.
15. **Nicoghosian, K., M. Bigras, D. Sankoff, and R. Cedergren.** 1987. Archetypical features in tRNA families. J. Mol. Evol. **26**:341–346.
16. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
17. **Palmer, J. D.** 1985. Comparative organization of chloroplast genomes. Annu. Rev. Genet. **19**:325–354.
18. **Platt, T.** 1986. Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. **55**:339–372.
19. **Reed, R. E., and S. Altman.** 1983. Repeated sequences and open reading frames in the 3′ flanking region of the gene for the RNA subunit of *Escherichia coli* ribonuclease P. Proc. Natl. Acad. Sci. USA **80**:5339–5363.
20. **Rogers, M. J., A. Steinmetz, and R. T. Walker.** 1984. A spiroplasma tRNA gene cluster. Israel J. Med. Sci. **20**:768–772.
21. **Rubnitz, J., and S. Subramani.** 1986. Extrachromosomal and chromosomal gene conversion in mammalian cells. Mol. Cell. Biol. **6**:1608–1614.
22. **Shapiro, J. A.** 1985. Mechanisms of DNA reorganization in bacteria. Int. Rev. Cytol. **93**:25–56.
23. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 137–138. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Vold, B. S.** 1985. Structure and organization of genes for transfer ribonucleic acid in *Bacillus subtilis*. Microbiol. Rev. **49**: 71–80.
25. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.
26. **Woese, C. R., W. G. Weisburg, C. M. Hahn, B. J. Paster, L. B. Zablen, B. J. Lewis, T. J. Macke, W. Ludwig, and E. Stackebrandt.** 1985. The phylogeny of purple bacteria: the gamma subdivision. Syst. Appl. Microbiol. **6**:25–33.