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Evolution of a tRNA Operon in Gamma Purple Bacteria

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Genomic DNA from eubacteria belonging to the gamma-3 subdivision of purple bacteria, as classified by Woese (C. R. Woese, Microbiol. Rev. 51:221–271, 1987), were probed with the *argT* operon of *Escherichia coli* encoding 5′-tRNA^{Arg}-tRNA^{His}-tRNA^{Leu}-tRNA^{Pro}-3′. The homologous operon from *Vibrio harveyi* was isolated and sequenced. Comparison of the five available sequences of this tRNA cluster from members of the families *Enterobacteriaceae*, *Aeromonadaceae*, and *Vibrionaceae* led to the conclusion that variations in different versions of this operon arose not only by point mutations but also by duplication and addition-deletion of entire tRNA genes. This data base permitted the formulation of a proposal dealing with the evolutionary history of this operon and suggested that DNA regions containing tRNA genes are active centers (hot spots) of recombination. Finally, since the operon from *V. harveyi* was not highly repetitive and did not contain tRNA pseudogenes, as in the *Photobacterium phosphoreum* operon, hybridization of genomic DNAs from different photobacterial strains with probes specific for the repeated pseudogene element was performed. We conclude that the phylogenetic distribution of the repetitive DNA is restricted to strains of *P. phosphoreum*.

A genome can be characterized by the identity or sequence of the genes which it encodes as well as by the arrangement of those genes. Gene sequences have been used with considerable success as probes to determine ancestral relationships (4, 9), while the other potential evolutionary indicator, gene arrangement, has not been exploited until very recently (D. Sankoff, R. Cedergren, and Y. Abel, Methods Enzymol., in press). In order to provide a coherent data base for the study of genome arrangement and evolution, we initiated a systematic study of tRNA gene organization among eubacteria. The choice of tRNA genes for our study was based on several criteria. First, tRNA genes are short, are repeated many times in the genome, and in bacteria, are often clustered under the control of a single promoter (tRNA operons). Therefore, even short DNA sequences of tRNA operons from different organisms could provide a number of observable gene rearrangements. Second, close to 1,000 tRNA sequences have been determined (25) and are available for comparison with the data that we generated in this study. Finally, tRNA molecules are key players in protein synthesis; knowledge of their gene numbers and distribution could offer a new insight into the origin and evolution of translation.

With the exception of the conserved tRNA genes that are present in some rRNA gene operons, the eubacterial tRNA gene arrangement is quite variable among different phyla. tRNA genes from *Bacillus subtilis* are highly clustered (27), whereas enterobacterial genes are dispersed (8). To bridge this phylogenetic gap, DNAs from various organisms that hybridize to the tRNA operon *argT* from *Escherichia coli* (13) are being studied in our laboratory. The sequence of this cluster is known in *E. coli* (13), *Salmonella typhimurium* (3), and *Aeromonas hydrophila* (12). The organization of this operon is identical in all three species, although the sequence differs in all instances. A partial sequence is also available from *Photobacterium phosphoreum* (11), but this DNA fragment shows little organizational similarity to *E. coli* and has been characterized as containing highly repetitive tRNA^{Pro} pseudogenes at its 3′ terminus (11).

In order to determine the distribution of the *Photobacterium* repetitive pseudogenes among other members of the family *Vibrionaceae* and their relation to the tRNA gene organization in *argT*, the isolation and sequencing of the same tRNA gene cluster from *Vibrio harveyi*, another marine bacterium, were undertaken. The sequence reported here establishes the presence of five tRNA genes in *V. harveyi*: 5′-tRNA^{Arg}-tRNA^{His}-tRNA^{Pro}-tRNA^{His}-tRNA^{Pro}-3′. It also establishes that the tRNA^{Pro} pseudogenes of the type found in *P. phosphoreum* is absent. In addition, the similarity of the tRNA gene order in *V. harveyi* and *P. phosphoreum* suggests that the previously uncharacterized upstream region of the *Photobacterium* cluster could contain additional tRNA genes. Here we report that the upstream region of the *Photobacterium* cluster contains a promoter followed by a tRNA^{Arg} gene and a tRNA^{His} gene. These new data, along with those published previously, allow a comparative analysis of this cluster of tRNA genes from *E. coli*, *S. typhimurium*, *A. hydrophila*, *V. harveyi*, and *P. phosphoreum*.

MATERIALS AND METHODS

Cultivation of strains and preparation of genomic DNA. The strains used in this study are indicated in Table 1. All strains were grown in a high-level salt medium as described previously (11). *V. harveyi* was grown at 20°C, *P. phosphoreum* was grown at 18°C, and *Photobacterium leiognathi* was grown at 26°C. DNA was extracted by the procedure described by Silhavy et al. (24).

Cloning and sequencing procedure. A preparative *Eco*RI digest of 20 μg of *V. harveyi* genomic DNA was electrophoresed through a 0.7% low-melting-temperature agarose gel. The region of the gel corresponding to 7 kilobases (kb) was excised and melted, and the DNA was purified as described previously (10). These fragments were ligated with the *Eco*RI-digested arms of λ gt10 (Promega Biotec, Madison, Wis.) following the conditions suggested by the manufacturer. The plaques were screened with labeled *argT* from *E. coli*. A large preparation of λ DNA was made from a

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TABLE 1. Strains used in this study

Strain	Source or reference
V. harveyi	ATCC 14126[a]
P. phosphoreum	ATCC 11040
P. phosphoreum NZ-11D	23
P. phosphoreum PJ-1a	P. V. Dunlap[b]
P. phosphoreum HE-1a	P. V. Dunlap[b]
P. leiognathi	ATCC 25521
P. leiognathi LR-1a	7
P. leiognathi LN-1a	7

[a] ATCC, American Type Culture Collection, Rockville, Md.
[b] P. V. Dunlap, Ph.D. dissertation, University of California, Los Angeles, 1984.

positive clone by the procedure described by Silhavy et al. (24), digested with different restriction enzymes, and subcloned into M13mp19. *Hae*III-generated fragments of 1.4 kb and *Sau*3A-generated fragments of 1.1 kb were partially sequenced in both directions by using the Sequenase enzyme with the kit supplied by U.S. Biochemical Corp. (Cleveland, Ohio).

In order to obtain the upstream sequence from *P. phosphoreum*, a synthetic probe complementary to the 5′ end of the sequenced portion was made: 5′-CATGCTGACTGG GAGATC-3′. A *Dra*I-generated fragment of 0.4 kb and a *Hae*III-generated fragment of 0.5 kb, as indicated by hybridization with the synthetic probe, were subcloned into M13mp19 and sequenced as described above.

Preparation of labeled probes and hybridization. The first probe (probe A) consisted of the sequence of the last $tRNA^{Pro}$ gene and the complete repetitive element segment of *P. phosphoreum*. This fragment originated from plasmid pPPS70 as described previously (12) and was prepared as described in that report (11). The purified fragment was labeled by using an oligolabeling kit from Pharmacia (Uppsala, Sweden) and [α-^{32}P]dCTP. The following synthetic DNA probes were made to the tRNA pseudogene sequences of *P. phosphoreum*: 5′-CTGATGTTAGGAATAGC-3′ for the 118-nucleotide element (probe B) and 5′-TTGGTTTA AAGCTAGA-3′ (probe C) for the 93-nucleotide element. These oligonucleotides were labeled by standard procedures by using the polynucleotide kinase from Pharmacia and [γ-^{32}P]ATP.

A total of 3 μg of genomic DNA of each sample was digested with *Sau*3A according to the procedure recommended by Pharmacia and run through a 1% agarose gel overnight. The gel was transferred to a nylon membrane (Amersham Corp., Arlington Heights, Ill.) by the alkaline procedure and hybridized by the protocol furnished by Amersham. Dextran sulfate was not included in the hybridization solution when oligonucleotides were used, to avoid a highly radioactive background. Also, the hybridization temperature was reduced to 42°C for the 118-nucleotide element-specific probe B and to 30°C for probe C. The washing conditions were also less stringent when oligonucleotides were used; i.e., washing was performed at room temperature with a solution containing 6× SSC (24) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate.

RESULTS

Cloning and sequencing of tRNA genes. Digestion of total DNA from *V. harveyi* with *Eco*RI gave one major fragment of about 7.0 kb which hybridized with *E. coli argT* DNA. The *V. harveyi* 7-kb DNA fragment was ligated with the *Eco*RI arms of phage λ gt10, and positive clones were again identified by hybridization with *E. coli argT*. The sequence of overlapping subclones is shown in Fig. 1A. From this sequence, the presence of five tRNA genes could be inferred based on their ability to fold into a canonical cloverleaf structure; however, no repetitive tRNA pseudogenes were found.

Although *V. harveyi* did not have the repetitive tRNA pseudogene structures like those observed in *P. phosphoreum*, the order 5′-$tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$ so resembled the order in *P. phosphoreum* that the presence of a $tRNA^{Arg}$ gene and a $tRNA^{His}$ gene in the uncharacterized upstream region of the *P. phosphoreum* operon had to be considered. This prospect did not seem likely previously (11), since the upstream DNA segment which was sequenced seemed to be too long to be intergenic. In order to isolate the region further upstream, a synthetic oligonucleotide corresponding to the 5′-terminal region of the sequenced fragment was made and used to probe genomic DNA. Indeed, the sequence of a 400-base-pair (bp) *Dra*I fragment (Fig. 1B) demonstrated the presence of two tRNA genes with a putative promoter. A schematic representation of the possible secondary structures of this sequence and the clusters from *E. coli*, *A. hydrophila*, and *V. harveyi* is shown in Fig. 2.

Detailed analysis of the clusters. Table 2 shows the number of differences between the tRNA genes of each species. For $tRNA^{Leu}$, there were 11 differences between *A. hydrophila* and both *E. coli* and *S. typhimurium*. More than half of these differences fell into the extra arm. For $tRNA^{Arg}$, the matrix shows that there were fewer differences between *E. coli* and *A. hydrophila* than there were between *P. phosphoreum* and *V. harveyi*. On the other hand, for $tRNA^{Pro}$ and $tRNA^{His}$, there were virtually as many differences between *A. hydrophila* and *E. coli* as there were between *A. hydrophila* and *P. phosphoreum* or *V. harveyi*. Table 2 also gives the total number of differences for the three common tRNA genes $tRNA^{Arg}$, $tRNA^{His}$, and $tRNA^{Pro}$. These values corroborate the phylogenetic relationships that have been determined between these organisms previously (2, 9), and they demonstrate that the difficulty in relating organisms based on single tRNA sequences described above is significantly reduced, if not eliminated, when several tRNA gene sequences are considered.

Figure 3A shows the alignment of the promoter region of the five species studied here. These assignments have been validated in *E. coli* (13), *S. typhimurium* (3), and *A. hydrophila* (unpublished data) by in vitro transcription assays. For *V. harveyi* and *P. phosphoreum*, however, this has not been shown explicitly, although the sequences are in good agreement with the consensus sequence for eubacterial promoters (−35 element, TTGACA; −10 element, TATAAT; these elements were separated by 17 bp) (17). Just downstream from the −10 box was a GC-rich discriminator element which is thought to be common to all genes under stringent control in *E. coli* (16). A tract of at least five GC residues was found close to the transcription start point in all five organisms, suggesting a similar function in close relatives of *E. coli*. In addition, the *E. coli* and *S. typhimurium* sequences were complementary to a region located 2 nucleotides downstream from the −35 box. This feature is found in several other *E. coli* tRNA gene promoters and may function to modulate gene expression (8). Its presence in some relatives of *E. coli* (*S. typhimurium*) and its absence in others (*A. hydrophila*, *V. harveyi*, and *P. phosphoreum*) could be

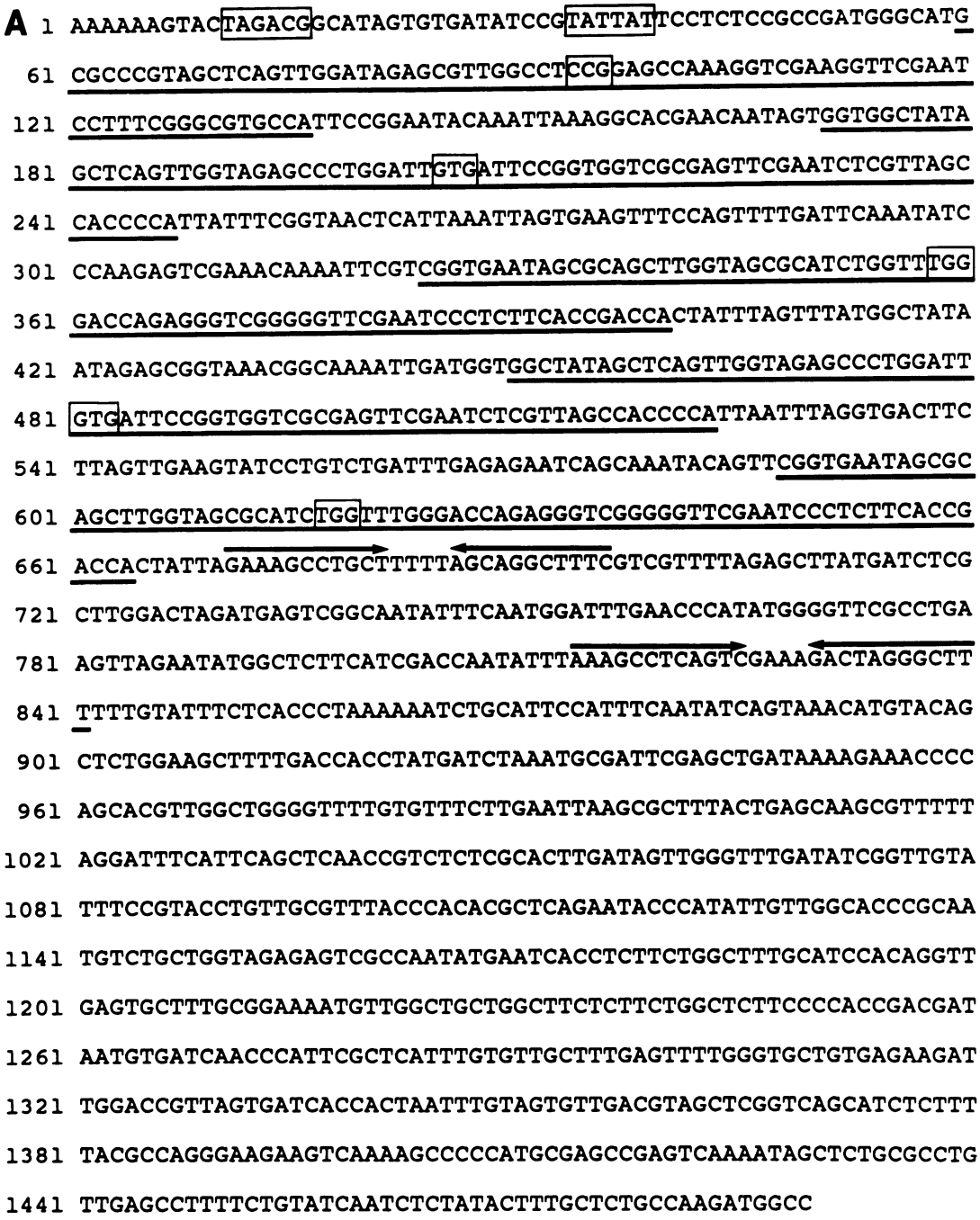

FIG. 1. (A) DNA sequence of the cluster isolated from *V. harveyi*. The promoter elements are boxed, and each tRNA gene sequence is underlined and its anticodon is boxed. The putative terminator sequences are indicated with arrows. (B) DNA sequence of the cluster from *P. phosphoreum*. The first 246 bp at the 5′ terminus is reported here for the first time, while the remaining sequence was published previously (11). For the entire sequence, see reference 11.

related to the gene copy number of one or all of the tRNAs present in the operon. In particular, $tRNA^{His}$, which is implicated in the regulation of the histidine biosynthetic pathway, has only one gene in *S. typhimurium* (3) and *E. coli* (26); and it might be very important for the tight regulation of this unique gene to link the $tRNA^{His}$ concentration to growth rate-dependent control.

The alignment of the presumptive [*rho*]-independent terminator segments is shown in Fig. 3B. Each segment is characterized by a GC-rich stem followed by a string of thymidines (21). In *E. coli* (13), *S. typhimurium* (3), *A. hydrophila* (unpublished data), and *P. phosphoreum* (11), an in vitro transcription assay showed that termination occurs in this region. No transcription experiments have been done with the operon from *V. harveyi*; however, we note two possible hairpin structures 3′ to the last tRNA gene. One located 10 nucleotides from the gene contains a GC-rich stem, a 4-nucleotide loop, and 3 consecutive T residues at the end. The other is found 150 bp downstream from the last gene and possesses a GC-rich stem, a loop of 4 bp, and a

FIG. 1—*Continued*

stem followed by 6 T residues. This latter hairpin is very similar to the *Photobacterium* terminator (Fig. 3B). Within this same region from position 790 to position 994, a potential open reading frame that has the capacity to code for a protein of 68 amino acids was observed. This open reading frame overlapped the second possible hairpin structure. By using FASTA (19), the hypothetical protein did not show significant similarity to any protein in the current National Biomedical Research Foundation protein data bank.

Comparison of intergenic spacers. Since intergenic spacers are generally less conserved than their flanking genes are, analysis of these spacers could provide important clues to decipher the gene rearrangements that occurred during the divergence of these species. Table 3 gives the number of nucleotides in each intergenic spacer. Corresponding spacers were aligned by using the FASTA/Align program (19). In *E. coli* and *S. typhimurium*, all spacers in corresponding positions were aligned with 76 to 88% sequence identity by using two to three gaps. The first and second spacers (between $tRNA^{Arg}$ and $tRNA^{His}$ and between $tRNA^{His}$ and $tRNA^{Leu}$) in *A. hydrophila* were aligned with the corresponding *E. coli* or *S. typhimurium* spacers with an average of 50% sequence identity with two to four gaps, while the last spacer (between $tRNA^{Leu}$ and $tRNA^{Pro}$) showed only 30% sequence identity with either *E. coli* or *S. typhimurium*. In *P. phosphoreum* and *V. harveyi*, only the first spacer between the $tRNA^{Arg}$ and $tRNA^{His}$ genes could be aligned. The best alignment provided 55.9% identity if two gaps were allowed (Fig. 4A). The others were diverse; for example, one spacer of 8 nucleotides in *P. phosphoreum* corresponded to a spacer of 46 nucleotides in *V. harveyi* (Table 3).

Only the first spacer between $tRNA^{Arg}$ and $tRNA^{His}$ genes was demonstrably related in all five species, and the level of similarity between them was in accordance with the relationship between the organisms based on 16S rRNA catalog (2, 9). This spacer in *A. hydrophila* showed 44.8% positional identity with three gaps when it was aligned with the *E. coli* spacer and 40.8% identity with three gaps when it was aligned with the *V. harveyi* spacer.

Besides the comparison of spacers between species, comparison of spacers within the same cluster could provide information on the origin of the repeated $tRNA^{His}$-$tRNA^{Pro}$ genes in *P. phosphoreum* and *V. harveyi*. The sequence identity of these repeated genes suggests that they originated from a tandem duplication. If this were the case, some similarity should be found in the spacers. A comparison of the spacer between the $tRNA^{His}$ and $tRNA^{Pro}$ genes in *V. harveyi* (see spacers 2 and 4 in Fig. 6) by using the program FASTA/Align permitted an alignment of 54.5% identity if five gaps were allowed (Fig. 4B). While the level of similarity of this region was significant in *V. harveyi*, the complete absence of similarity in *P. phosphoreum* did not support an origin by tandem duplication.

Phylogenetic distribution of the repeated pseudogene element. The absence of the repeated tRNA pseudogene element in *V. harveyi* prompted us to examine different strains of *P. phosphoreum* as well as strains from the only other reported species of the genus *Photobacterium*, *P. leiognathi*, for the presence of the repeated element. Figure 5 shows gel autoradiograms obtained with different labeled probes. Probe A, which was a segment that contained the full repeated element along with an entire $tRNA^{Pro}$ gene, hybridized strongly to every strain of *P. phosphoreum* (Fig. 5A, lanes 1 through 4) and to a lesser extent with the strains of *P. leiognathi* (Fig. 5A, lanes 5 through 7). It must be kept in mind, however, that this probe hybridized as well with the other $tRNA^{Pro}$-$tRNA^{His}$ gene cluster described previously (10) (Fig. 5A, lane 1, band a) and, thus, is not necessarily indicative of the presence of the repetitive segment in this operon. The single band obtained with the DNA from *V. harveyi* (Fig. 5A, lane 8) corresponded to the operon characterized here and undoubtedly originated from the similarity of $tRNA^{Pro}$ genes from *P. phosphoreum* and *V. harveyi*.

Using a synthetic probe (probe B) complementary to the

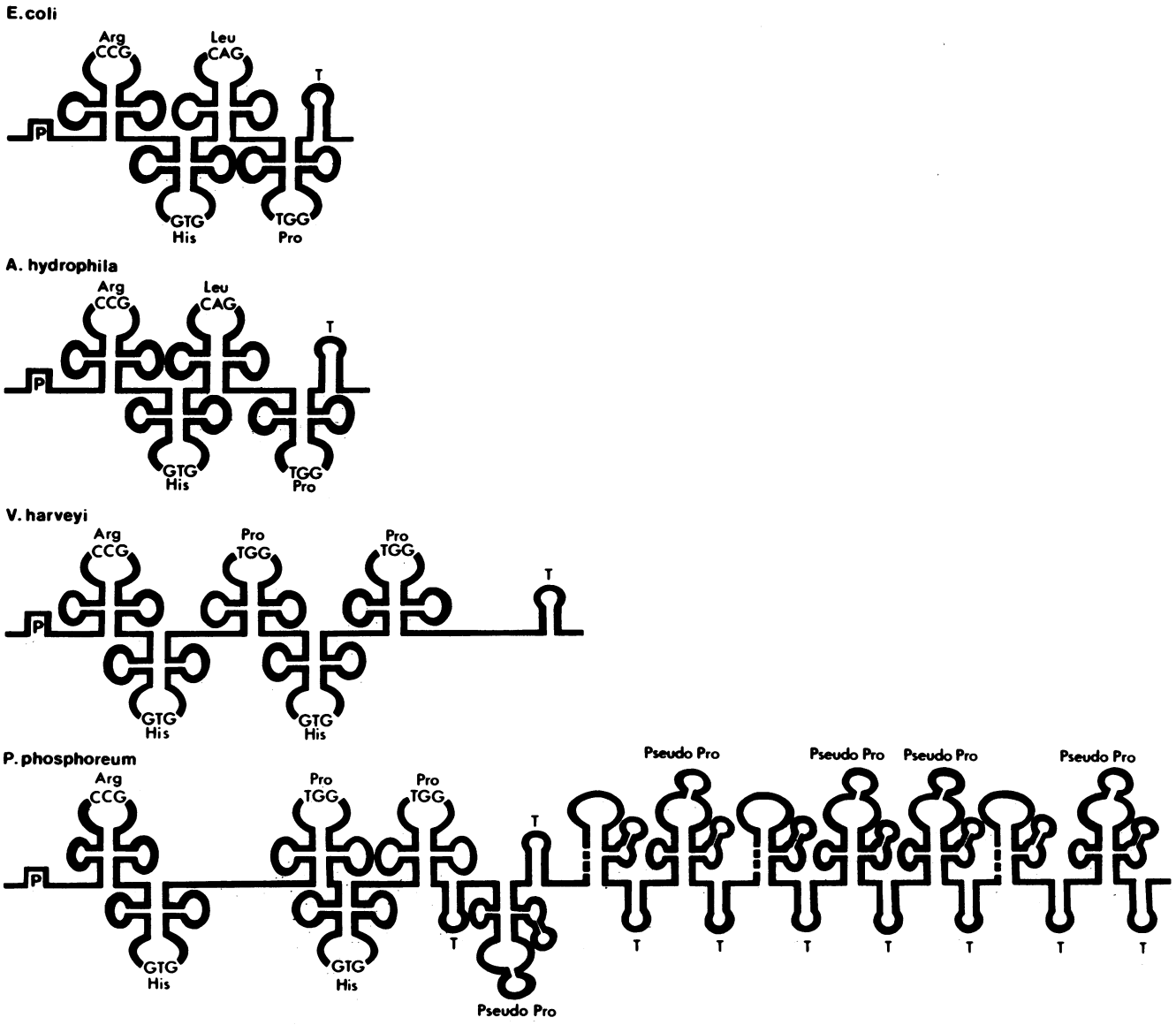

FIG. 2. Schematic drawing of the possible secondary structures of the sequenced fragments from *E. coli*, *A. hydrophila*, *V. harveyi*, and *P. phosphoreum*.

insertion sequence described previously (11), we obtained a hybridization signal only with strains of *P. phosphoreum* (Fig. 5B, lanes 1 through 4). Another synthetic probe (probe C) complementary to the 93-element sequence described previously (11) hybridized only with strain ATCC 11040 and was specific for the cluster from which it came (Fig. 5C, lane 1, band b, as in Fig. 5A). Taken together, these results indicate that strain-specific variations occur in *P. phos-*

TABLE 2. Difference matrix for tRNA gene sequences

Species	No. of differences[a]																				Total no. of differences				
	$tRNA^{Arg}$					$tRNA^{His}$					$tRNA^{Leu}$					$tRNA^{Pro}$									
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
E. coli		2	2	9	9		0	7	6	10		0	11	ND[b]	ND		0	11	10	11		2	20	25	30
S. typhimurium			2	9	8			7	6	10			11	ND	ND			11	10	11			20	25	29
A. hydrophila				10	8				11	7				ND	ND				9	10				30	25
V. harveyi					4					6					ND					1					11
P. phosphoreum																									

[a] Numbers in the heads indicate the following organisms: 1, *E. coli*; 2, *S. typhimurium*; 3, *A. hydrophila*; 4, *V. harveyi*; 5, *P. phosphoreum*.
[b] ND, Not determined.

A

SPECIES	-35 ELEMENT	-10 ELEMENT DISCRIMINATOR
E.coli	AAAAAGGGACTAGACAGAGGGGTGGGAAGTCCGTATTATCCACCCCCGCAACGGCGCTAA	
S.typh	AAAAAGGCACTGGACAGCGGGGTGTGAAGTCCGTATTATCCACCCCCGCAACGGCGCTAA	
A.hydr	AAAAAAATAGTAGACAAGGTGGGTCCCCATCATTAATATTGCGCCCCGTTCGACAGCGTA	
V.harv	AAAAAAGTACTAGACGGCATAGTGTGATATCCGTATTATTCCTCTCCGCCGATGGGCAT	
P.phos	AAAAAAACACTTGCCGAGTCGCGTTCTGATCCGTAATATTCACCCCGTCGATAGGGCAAG	

B

SPECIES	SPACE (BP)	TERMINATION REGION
E.coli	0	ATTTTGAACCCCGCTTCGGCGGGGTTTTTTG
S.typh.	0	ATTTTGAACCCCGCTTCGGCGGGGTTTTTTG
A.hydr.	4	CTGAAAAACCCCGCTCAGGCGGGGTTTTTTG
V.harv.	3	TTAGAAAGCCTGCTTTTTAGCAGGCTTTCGTTCGTTTT
V.harv.	146	TTTAAAGCCTCAGTCGAAAGACTAGGGCTTTTTTG
P.phos.	5	TAAGAAAACCGTATCGAAAGATACGGTTTTTTTTC

FIG. 3. (A) Sequence of the promoter region. The −35 element, the −10 element, and the GC-rich discriminator are underlined. The first gene starts at the end of the sequence. (B) Sequence of the termination region of each cluster. The distances (in base pairs [BP]) between the last $tRNA^{Pro}$ gene and the beginning of the sequence indicated are listed in the column labeled space.

phoreum and that the repeated pseudogene element evolved quite recently, even though comparison of different copies of the element showed the fixation of some mutations (11).

DISCUSSION

Analysis of the operon sequences from five organisms allowed us to make certain hypotheses concerning the evolutionary pathway that has led to the current organization (Fig. 6), and in this regard, the intergenic sequences are particularly meaningful. The sequence between $tRNA^{Arg}$ and $tRNA^{His}$ in all species conserved enough residual similarity to suggest that this segment has not been involved in major rearrangements. The changes observed in this spacer reflect the time since the divergence of these species. Within the *Vibrio* operon, the similarity observed between the repeated spacers, i.e., spacer 2 and spacer 4 in Fig. 6, was less than that observed between the $tRNA^{Arg}$ and $tRNA^{His}$ spacers of *V. harveyi* and *P. phosphoreum* (compare Fig. 4A and B); therefore, the putative duplication of the $tRNA^{His}$ and $tRNA^{Pro}$ genes would have occurred before the divergence of the two species (Fig. 6). This hypothesis is also the logical consequence of the fact that such a duplication would not have arisen independently in both *V. harveyi* and *P. phosphoreum*. However, the lack of any similarity between the spacers of the repeated element in *P. phosphoreum* raises the question of the origin and derivation of the spacers.

TABLE 3. Spacer length comparison

Species	Spacer length (bp) between the following tRNA genes:					
	Arg-His	His-Leu	Leu-Pro	His-Pro	Pro-His	His-Pro
E. coli	57	20	42			
S. typhimurium	53	20	42			
A. hydrophila	49	20	71			
V. harveyi	34			76	46	64
P. phosphoreum	34			161	8	53

In trying to explain the lack of similarity in the spacer region, it must be recalled that the *Photobacterium* genome has a second gene cluster containing two $tRNA^{His}$ and six $tRNA^{Pro}$ genes (10). Furthermore, a 304-nucleotide segment of this cluster demonstrated 99% positional identity with the $tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$ region considered to be duplicated. Even though the origin of the second cluster is obscure, it could have served as a donor of the present $tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$ sequence in the original operon (Fig. 7). By this mechanism the original intergenic sequence, which was derived from the duplication of the $tRNA^{His}$-$tRNA^{Pro}$ segment, would be replaced by a sequence which would show no similarity (Fig. 6). This conversion must have evolved recently because only three transitions have occurred in the spacers of the homologous segment.

Concerning the fate of $tRNA^{Leu}$ gene, its gain in the *A. hydrophila-E. coli* lineage or loss in the vibrios must have

A

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T  TCCGGAATACAAATTAAAGGC ACGAACAATAGT        V.harveyi
*  *****             *****  ** ******
TCATCCGGTCGTATTGGCAAAGGTTACAAACAAT           P.phosphoreum
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B

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1 TTA TTTCGGTAACTCATTAAATTAGTGAAGTTTCCAGTTTTGATT
  *** *** *** ***  ***       ****** *** **  *****
2 TTAATTTAGGTGACTTCTTA     GTTGAAGTATCCTGTC TGATT


1 CAAATATCCCAAGAGTCGAAACAAAATTCGT
    *         *** **   * ** *    *
2 TGAG        AGAATCAGCA AATACAGTT
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FIG. 4. (A) Comparison of the 34-nucleotide spacer between tDNA^{Arg} and tDNA^{His} of *V. harveyi* with its 34-nucleotide counterpart from *P. phosphoreum*. (B) Comparison of the duplicated spacer between tDNA^{His} and tDNA^{Pro} from *V. harveyi*. Sequence 1 is from position 248 to position 323, and sequence 2 is from position 524 to position 587 in Fig. 1A. Asterisks indicate identical positions.

preceded the duplication of the tRNA^{His}-tRNA^{Pro} region, since it is unlikely that either event happened twice (Fig. 6). In other words, the common ancestor of this operon in the five species examined here consisted of single genes in the same order as those in the families *Aeromonadaceae* and *Enterobacteriaceae* (Fig. 6). Preliminary results on the hybridization of the restricted genome of *Pseudomonas fluorescens* with synthetic probes suggest that the tRNA^{Leu} gene

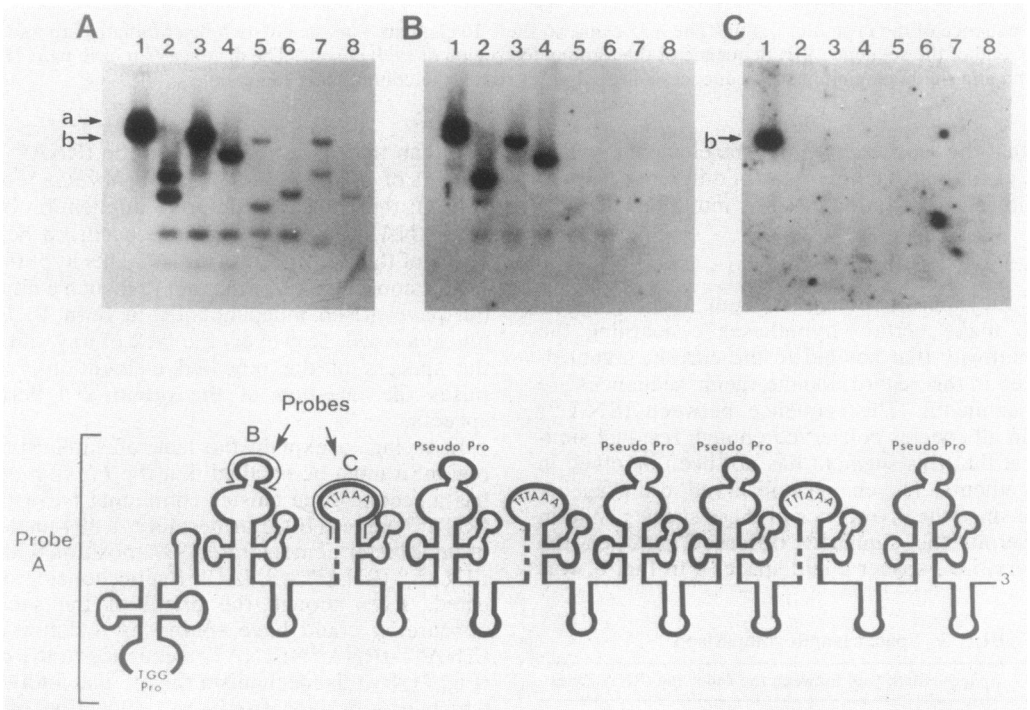

FIG. 5. Hybridization of *Sau*3A-restricted genome from *P. phosphoreum* ATCC 11040 (lanes 1), *P. phosphoreum* NZ-11D (lanes 2), *P. phosphoreum* HE-1a (lanes 3), *P. phosphoreum* PJ-1a (lanes 4) *P. leiognathi* ATCC 25521 (lanes 5), *P. leiognathi* LN-1a (lanes 6), *P. leiognathi* LR-1a (lanes 7), and *V. harveyi* ATCC 14126 (lanes 8) with different probes. (A) The probe was the fragment containing a tRNA^{Pro} gene followed by a terminator and the eight repeated elements, as shown in the diagram below the autoradiograms. (B) the probe was a synthetic oligonucleotide corresponding to the section indicated among the repeated elements. (C) the probe was a synthetic oligonucleotide corresponding to the section indicated in the diagram below the autoradiograms. The arrows in panel A indicate the second cluster of 2.6 kb (a) and part of the cluster which corresponds to probe A (b).

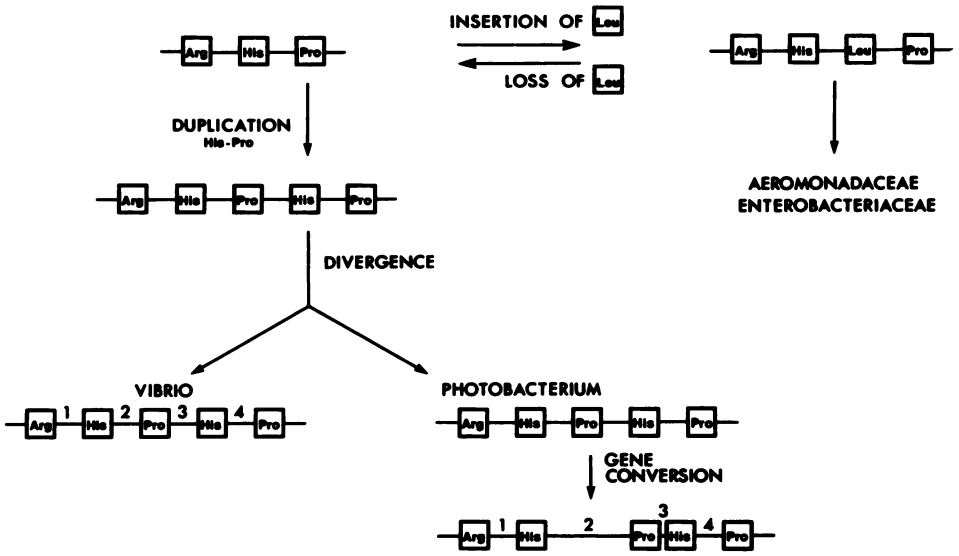

FIG. 6. Possible evolutionary scheme according to the results obtained in this study. Each box represents a tRNA gene. The numbers present on the *V. harveyi* and *P. phosphoreum* clusters indicate the spacers referred to in the Discussion section.

is linked to the other tRNA genes, as in aeromonads and enterobacteria. The eventual linkage of the $tRNA^{Leu}$ gene with those of $tRNA^{Arg}$ and $tRNA^{His}$ in *Pseudomonas fluorescens* would firmly establish the loss of the $tRNA^{Leu}$ gene in the branch leading to the family *Vibrionaceae*.

The results dealing with the presence of the repetitive pseudogenes in *P. phosphoreum* suggest that DNA without any apparent function can be maintained and even amplified in a eubacterial genome. The polymorphism observed between the different natural isolates suggests that the turnover mechanisms defined by Dover (5) could be responsible for the observed variations in the photobacterial population. Polymorphism within the *phoA* gene of different *E. coli* strains has been reported (6), and it was probably generated through the genetic exchange of small segments of DNA between natural isolates. In this case, however, we believe that dynamic processes of the genome itself (DNA turnover), such as unequal crossing over, gene conversion, and replication slippage, were the mechanisms (1, 20) which accounted for the diversity observed among our isolates. This was suggested by the high recombinogenic potential generated by the nearly perfect repeated segment present in a single locus and between different loci in *P. phosphoreum*.

We compared tRNA gene operons that were isolated from closely related bacteria belonging to the gamma-3 subdivision of the purple bacteria (28). The results are in good agreement with the phylogenetic tree established by using the 16S oligonucleotide cataloging method (2, 9). Our results also indicate that *S. typhimurium* is more closely related to *E. coli* than *V. harveyi* is to *P. phosphoreum* and that *A. hydrophila* is more closely related to members of the family *Enterobacteriaceae* than it is to the lineage of *Vibrionaceae* (2). It seems that even in a short evolutionary space, major rearrangement can occur among tRNA genes. In this regard some tRNA genes may, in fact, be hot spots of recombination (22). However, this might not be true for all tRNA genes; the cluster analogous to *thrU* (*tufB*) from *E. coli* (14) has been isolated from *Pseudomonas aeruginosa* (15). Here, the four tRNA genes of the cluster were organized in a fashion identical to that in *E. coli*. It is possible that tRNA genes linked to protein genes and rRNA genes are more conserved for reasons that are not yet understood. On the

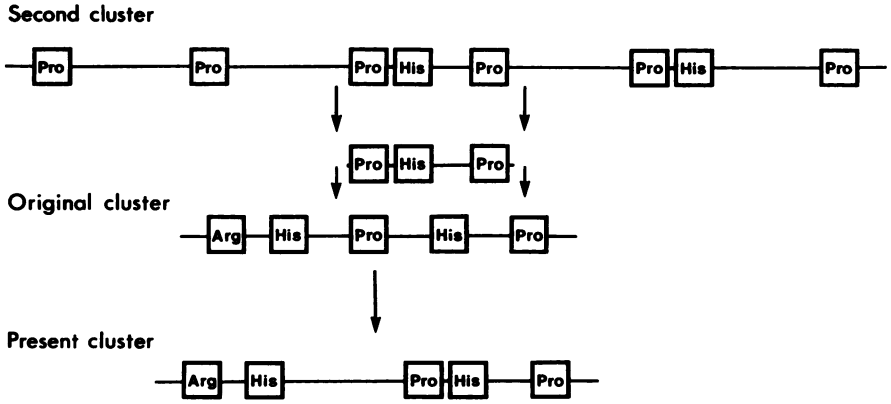

FIG. 7. Model for a gene conversion. Each box represents a tRNA gene. In this scheme the donor is the second cluster; the acceptor is a putative original cluster in which the segment $tRNA^{His}$-$tRNA^{Pro}$ has been duplicated in a ancestor of all *Vibrio* species. The gene conversion event resulted in the loss of the original $tRNA^{Pro}$-$tRNA^{His}$-$tRNA^{Pro}$ segment.

other hand, clusters containing only tRNA genes could be under fewer constraints and freer to rearrange more rapidly. To obtain further information on the rearrangement of tRNA genes, a study is now in progress in which the *metT* operon from *E. coli* is used as a probe (18).

Finally, with the great interest generated recently by the idea of genome sequencing, we anticipate that in the next few years there will be an exponential growth in sequencing data and a resultant new appreciation of genomic fluidity. In that sense, the tRNA gene rearrangements reported here have opened only a small window on the complexity and the importance of genome organization.

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