Evolution of tRNAs and tRNA genes in *Acholeplasma laidlawii*

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ABSTRACT

The genes for 22 tRNA species from *Acholeplasma laidlawii*, belonging to the class *Mollicutes* (Mycoplasmas), have been cloned and sequenced. Sixteen genes are organized in 3 clusters consisting of eleven, three and two tRNA genes, respectively, and the other 6 genes exist as a single gene. The arrangement of tRNA genes in the 11-gene, the 3-gene and the 2-gene clusters reveals extensive similarity to several parts of the 21-tRNA or 16-tRNA gene cluster in *Bacillus subtilis*. The 11-gene cluster is also similar to the tRNA gene clusters found in other mycoplasma species, the 9-tRNA gene cluster in *M. capricolum* and in *M. mycoides*, and the 10-tRNA gene cluster in *Spiroplasma meliferm*. The results suggest that the tRNA genes in mycoplasmas have evolved from large tRNA gene clusters in the ancestral Gram-positive bacterial genome common to mycoplasmas and *B. subtilis*. The anticodon sequences including base modifications of 15 tRNA species from *A. laidlawii* were determined. The anticodon composition and codon-recognition patterns of *A. laidlawii* resemble those of *Bacillus subtilis* rather than those of other mycoplasma species.

INTRODUCTION

Mycoplasmas, wall-less prokaryotes, are grouped in the class *Mollicutes*, which contains six genera, *Acholeplasma*, *Anaeroplasm*, *Asteroleplasma*, *Mycoplasma*, *Spiroplasma* and *Ureaplasma*. They are phylogenetically related to Gram-positive eubacteria such as *Bacillus* spp. and *Clostridium* spp. (1–4), however, with the genome size much smaller. They are thus regarded as a degenerated form of Gram-positive bacteria. The G + C content of genomic DNA of all the mycoplasma species so far reported is very low, ranging from 25 to 42% (see for a review, ref. 5).

We have reported the sequences for the complete set of tRNA species (6,7) and their genes from *Mycoplasma capricolum* (genus

Mycoplasma) (8). Reflecting small size of the genome, *M. capricolum* contains only 30 tRNA genes encoding 29 tRNA species with 28 different anticodons, which are the smallest in number in all the known genetic systems except for mitochondria. The anticodon composition and codon-anticodon recognition patterns of *M. capricolum* are unique in many ways as compared with those of other bacteria such as *E. coli* and *B. subtilis*. For example, most of four synonymous codons in family-boxes are read by a single anticodon UNN, U unmodified, with deletion of anticodon GNN and CNN (7,9,10), and many non-obligate CNN anticodons in two-codon sets are absent. As a result, most of the synonymous set of codons are translated each by a single tRNA species. The amino acid assignment for two codons deviates from the universal genetic code: codon UGA from stop to Trp (11), and codon CGG from Arg to an unassigned (12), in accordance with the existence of anticodon UCA (Trp) and the absence of anticodon CCG (Arg), respectively. These characteristic features may be brought about by two evolutionary constraints, economizing the genome size and keeping the genomic G + C content low, that have been exerted on the mycoplasma genome during evolution (7,8).

Acholeplasma laidlawii also belongs to the class *Mollicutes*, (genus *Acholeplasma*). Phylogenetic analysis of rRNA sequences has shown that *Acholeplasma* has diverged from other genera, such as *Mycoplasma*, *Spiroplasma* and *Ureaplasma*, in early stage of the development of *Mollicutes* in a Gram-positive bacterial lineage (1–4,13). The genome size of *A. laidlawii* (about 1700kbp) is larger than that of *M. capricolum* (about 1000kbp), but is much smaller than that of *B. subtilis* (about 4200kbp). The G + C content of *A. laidlawii* DNA shows an intermediate value (32%) between those of *B. subtilis* (42%) and *M. capricolum* (25%). Thus, *A. laidlawii* would be an adequate organism to see the changes in tRNAs during evolution of *Mollicutes*. Here, we report the sequences of 21 tRNA genes and the anticodon sequences including modified nucleosides of 15 major tRNA species from *A. laidlawii*. The deduced gene organization and anticodon composition of the *A. laidlawii* tRNAs are compared with those of *B. subtilis* and *M. capricolum*, and a possible process of evolutionary changes in tRNAs of *Mollicutes* is discussed.

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MATERIALS AND METHODS

Culture of *A.laidlawii* cells

A.laidlawii [American Type Culture Collection 23206] cells were grown at 37°C in the medium containing 2.2% (w/v) PPLO broth (Difco), 1% (V/V) horse serum (Gibco), 0.2% (w/v) glucose, 400 units(U)/ml of penicillin G, and 50mM Tris-HCl (pH7.6). Cells were collected at late log-phase, washed with PBS (Phosphate buffered saline: 0.14M NaCl, 2.7mM KCl, 8mM Na₂HPO₄, 1.1mM KH₂PO₄) and stored at -70°C.

Preparation of total tRNA

A.laidlawii tRNAs were prepared by the direct phenol extraction method (14). The tRNA was deacylated by incubation in 1M Tris-HCl (pH9.0) at 37°C for 2 h and in 0.5M lysine (pH8.8) at 37°C for 2h. Deacylated tRNA was precipitated with ethanol, dissolved in TE-buffer (10mM Tris-HCl, 1mM EDTA, pH8.0) and dialyzed against TE-buffer.

Selective labelling of isoacceptor tRNAs

Selective labelling of isoacceptor tRNAs was performed according to the method of Traboni *et al.* (15), with slight modifications as described by Andachi *et al.* (7). For the preparation of hybridization probe, the 3'-end-labelling was performed with 50μCi of [5'-³²P]pCp (3000 Ci/mmol) and 50 unit of phage T4 RNA-ligase in the reaction mixture (20μl), as described by England *et al.* (16). Labelled tRNAs were separated by 12% polyacrylamide gel electrophoresis, and each isoacceptor species was eluted from the gel.

Hybridization

The 3'-end-labelled total tRNAs were prepared as described above, separated from contaminating 5S rRNA and free pCp by 12% polyacrylamide gel electrophoresis and eluted from the gel. The tRNAs were collected by ethanol precipitation together with 5μg carrier *E.coli* ribosomal RNA (Sigma) and dissolved in 20μl 5×SSC.

The DNA-blotted nitrocellulose filter was incubated in a solution containing 5×SSC, 5×Denhardt's solution, 0.5% SDS, 50% formamide and ³²P-labelled tRNA at 42°C for 15 h. The filter was washed with 5×SSC, 0.1% SDS at 42°C three times, dried and exposed to an X-ray film.

Figure 1. Distribution of tRNA genes in the *Acholeplasma laidlawii* genome. Southern hybridization of *Xba*I-digested *A.laidlawii* total DNA and total tRNAs labelled with [³²P]pCp. Correspondence of the hybridized band and the cloned tRNA genes or clusters are indicated.

Cloning and sequencing

Total DNA was digested with *Xba*I and separated by 0.7% agarose gel electrophoresis. The DNA fragments with appropriate size were recovered from the gel, ligated into *Xba*I site of the plasmid vector Bluescript KS⁺ (Stratagene) and transformed to *E.coli* JM109 or DH5α. Colony hybridization was performed to select the cells which carried the recombinant plasmid including tRNA genes. Some tRNA genes were selected with tRNA probes labelled by selective labelling method. The nucleotide sequences were determined as described previously (8).

RNA sequencing

The nucleotide sequences of isolated tRNAs were determined by the post-labelling method of Kuchino *et al.* (17) as previously described (7,18).

Materials

All restriction endonucleases, T4 RNA-ligase, ExoIII, ExoVII nucleases were purchased from Takara-Shuzo Co. Ltd. (Kyoto, Japan); Radioactive compounds (³²P and ³⁵S) were from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). For sequencing DNA, 'Sequenase' kit of Toyobo Co., Ltd. (Osaka, Japan) was used.

RESULTS

Organization of tRNA genes

Southern hybridization of the *Xba*I-digested total DNA and ³²P-labelled total tRNAs of *A.laidlawii* revealed at least 13 bands with the size ranging from 2.1 to 15kbp (Fig 1). Among them, 10 DNA fragments corresponding to the bands, 2.1, 2.4, 3.0, 3.2, 4.0, 5.5, 6.2, 6.3, 7.8, and 8.8 kbp, were cloned and the sequences including respective tRNA genes and their flanking regions were determined. Two DNA fragments, 3.4 and 6.2 kbp, contained the same sequence including a cluster of genes for 11 tRNAs. The sequence of the gene cluster in the 3.4 kbp fragment was fully determined, and most parts of the gene cluster in the 6.2 kbp fragment were also sequenced. These showed that the two clusters were identical each other not only in their gene organization but also in the sequences of tRNA genes and spacer regions so far determined. Probably, the two clones were derived

Figure 2. The organization of tRNA genes in *A.laidlawii*. The gene arrangements in the 9 clones including tRNA genes are schematically shown. The tRNA genes are indicated by box with the specifying amino acid and the anticodon. Abbreviations: P, promoter; T, terminator. Numbers above the lines indicate the number of base pairs in the spacers.

from the same genomic DNA region due to incomplete *Xba*I digestion or a local sequence heterogeneity at one of the *Xba*I-sites in the population of *A. laidlawii* DNA. The 7.8 kbp fragment contained a cluster of three tRNA genes, tRNA^{His}(GTG)-tRNA^{Gln}(TTG)-tRNA^{Leu}(TAG), and the 8.8 kbp fragment contained a 2-gene cluster for tRNA^{Ser}(GCT)-tRNA^{Glu}(TTC). The other 6 fragments included each a single tRNA gene. The sequence of tRNA^{Trp}(CCA) gene, which located in the 6.3 kbp fragment, was already reported (19). Altogether, the sequences of 22 tRNA genes in nine different clones were determined. The sequence data will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers from X61061 to X61068.

Fig. 2 summarized the organization of tRNA genes and clusters in the nine clones. The 11-tRNA gene cluster and tRNA^{Asn}(GTT) gene located at the 3'-downstream regions of rRNA gene clusters (rRNA operons), respectively (see Fig. 2). They seem to be cotranscribed with each rRNA operon, because the spaces between the 3'-end of 5S rRNA and the 5'-end of the tRNA gene are relatively short (29 bp and 58 bp, respectively), without containing promoter- and/or terminator-like sequences.

Figure 3. Sequences of anticodons and their neighboring regions of 15 tRNA species from *A. laidlawii* shown in clover-leaf form. Abbreviations used for modified nucleosides are: i⁶A, N⁶-isopentenyladenosine; t⁶A, N-((9-D-ribofuranosylpurine-6-YL)-carbamoyl)threonine; m⁶A, N⁶-methyladenosine; I, inosine; ψ, pseudouridine; cmnm⁵U, 5-carboxymethylaminomethyluridine; m⁵U, 5-methoxyuridine; m¹G, 1-methylguanine; N, undetermined nucleotide; *U, uridine with unidentified modification.

All the other tRNA genes and clusters were preceded each by a promoter-like sequence resembling *E. coli* -35 and -10 promoter consensus sequence at 5'-upstream, and followed by rho-independent terminator-like structure, consisting of a dyad-symmetrical sequences and T-cluster at the 3'-downstream region, suggesting that they consisted of a single transcription unit (operon). The 3'-terminal CCA was all encoded in DNA.

Anticodon sequences

Total tRNAs from *A. laidlawii* were separated by 12% polyacrylamide gel electrophoresis in the presence of 7M urea. After staining by ethidium bromide, major tRNA bands were eluted from the gel, and further purified by 8% polyacrylamide gel electrophoresis. The nucleotide sequences including modified nucleotides around the anticodon position from 15 isolated tRNA species were determined by post-labelling method (Fig. 3). The sequences of 12 tRNAs, tRNA^{Ala}(UGC), tRNA^{Ser}(UGA), tRNA^{Leu}(UAA), tRNA^{Leu}(UAG), tRNA^{His}(GUG), tRNA^{Arg}(UCU), tRNA^{Asp}(GUC), tRNA^{Thr}(UGU), tRNA^{Lys}(CUU), tRNA^{Gln}(UUG), tRNA^{Trp}(CCA), and tRNA^{Val}(UAC), agreed with the DNA sequences of tRNA genes found in this study. The sequences corresponding to the other 3 tRNAs, tRNA^{Arg}(ICG), tRNA^{Thr}(GGU) and tRNA^{Tyr}(GUA), were not included in the tRNA genes determined.

The 5'-terminal nucleoside (the first position) of anticodons was determined by two-dimensional thin-layer chromatography as described previously (7,18). Among seven anticodons belonging to family-boxes, the first nucleosides of all the five UNN-type anticodons, UAG(Leu), UAC(Val), UGA(Ser), UGU(Thr) and UGC(Ala), were modified to 5-methoxyuridine (m⁵U), a derivative of 5-hydroxyuridine(20). The other two anticodons were GGU(Thr), the first nucleoside G unmodified, and ICG(Arg) (I: inosine). There were three UNN anticodons in NNR-type (R: A or G) two-codon sets: 5-carboxymethylaminomethyl-2'-O-methyluridine (cmnm⁵Um) was used in the first position of anticodon UAA(Leu); 5-carboxymethylaminomethyluridine (cmnm⁵U) was in

Figure 4. Labelling of *A. laidlawii* tRNAs specific for a single amino acid. The (3'-³²P)-labelled isoacceptor tRNAs for several amino acids were separated by electrophoresis on a 12% polyacrylamide/7 M-urea gel and autoradiographed. The left lane shows the total tRNAs. Next two lanes show control experiment carried out without enzyme and without amino acid.

UAU(Arg); the modification in UUG(Gln) could not be identified. The G in the GNN anticodons in NNY-type (Y:U or C) two-codon sets, GUA(Tyr) and GUC(Asp), was unmodified. The C in the three CNN anticodons, CAA(Leu), CUU(Lys) and CCA(Trp), was all unmodified.

Four types of modified nucleosides, N6-isopentenyladenosine (i^6 A), N-((9-beta-D-ribofuranosylpurine-6-yl)-carbamoyl)-threonine (t^6 A), N6-methyladenosine (m^6 A) and 1-methylguanosine (m^1 G), were identified at position 37, which is adjacent to the 3'-terminal of the anticodon. The i^6 A existed in tRNA^{Leu}(UAA), tRNA^{Ser}(UGA), tRNA^{Trp}(CCA) and tRNA^{Tyr}(GUA), all of which recognize codons starting with A, and t^6 A in tRNA^{Arg}(UCU), tRNA^{Lys}(CUU), tRNA^{Thr}(UGU) and tRNA^{Thr}(GGU) translating ANN codons. The m^6 A was found in tRNA^{Ala}(UGC), and m^1 G in tRNA^{Arg}(ICG), tRNA^{His}(GUG) and tRNA^{Leu}(UAG) for CNN codons. The 37 position of tRNA^{Gln}(UUG) and tRNA^{Asp}(GUC) was unmodified A.

The number of isoacceptor tRNAs

The number of isoacceptor tRNAs for each of several amino acids was estimated by selective labelling method (15). Fig. 4 shows the radioactive bands of isoacceptors for each of 14 amino acids separated by polyacrylamide gel electrophoresis. The patterns for the other 6 amino acids could not be obtained because the aminoacylation activities for these amino acids in the S100 fraction of *A.laidlawii* were very low.

The number of detected isoacceptor tRNAs was at least four for Leu, three each for Ser and Arg, two each for Gly, Ala, Lys and Thr, and one each for His and Trp. Two bands appeared in Tyr lane: partial sequencing analysis showed that they were an identical tRNA species. It should be noted that there are at least two isoacceptors each for Ala, Thr and Gly, which belong to family-boxes.

DISCUSSION

Evolution of tRNA genes

Present study has revealed the structures of 22 tRNA genes in nine different DNA fragments from *A.laidlawii*. Among them, 16 genes are arranged in the clusters containing 11, 3 and 2 tRNA genes, respectively (Fig. 2). The organization and sequences of all the 30 tRNA genes in *M.capricolum* (7,8), and those of 51 tRNA genes in *B.subtilis* have been reported (21). Since these three species are phylogenetically related each other, the organizations of the tRNA genes are compared between them.

In *B.subtilis*, major tRNA genes are organized in the four clusters, including 21 tRNA genes (21-gene cluster), 16 genes (16-gene cluster), 6 genes (6-gene cluster) and 4 genes (4-gene cluster), respectively, and some in the spacers of two rRNA operons (22–27). The tRNA gene organization in *A.laidlawii* shows similarity to that in the 21- and 16-gene clusters in *B.subtilis*. As shown in Fig 5, the order of genes from tRNA^{Val}(TAC) to tRNA^{Lys}(TTT) and from tRNA^{Ala}(TGC) to tRNA^{Phe}(GAA) in the 11-gene cluster is identical to that of portions of the *B.subtilis* 21-gene cluster. It is known that the gene order in the 9-tRNA gene clusters found in *M.capricolum* (8) and *M.mycoides* (28), and that in the 10-tRNA gene cluster in *Spiroplasma meliferm* (29) are identical to a portion, from tRNA^{Arg}(AGC) to tRNA^{Phe}(GAA), of the 21-gene cluster in *B.subtilis*. The arrangement of seven genes from tRNA^{Ala}(TGC) to tRNA^{Phe}(GAA) in the 11-gene cluster in *A.laidlawii* is also

identical to the portions of the large gene clusters found in these *Mollicutes*, indicating that all these clusters share a common origin with the 21-gene cluster in *B.subtilis*. The *A.laidlawii* 11-gene cluster locates at the 3'-downstream region of 5S rRNA gene in one of the rRNA operons. The *B.subtilis* 21-gene cluster also locates at the 3'-downstream of an rRNA operon (*rrnE*). Thus, it is clear that the 11-tRNA gene cluster in *A.laidlawii* has derived from a large tRNA gene cluster similar to the present *B.subtilis* 21-gene cluster by deleting or translocating several tRNA genes during evolution. The *B.subtilis* 16- and 6-gene clusters also locate at the 3'-downstream regions of *rrnD* and *rrnB*, respectively, and the first (5'-proximal) tRNA gene in the both clusters is tRNA^{Asn}(GTT). Location of *A.laidlawii* tRNA^{Asn}(GTT) gene at the 3'-downstream of the other rRNA operon suggests that the gene shares the common origin with one of the clusters in *B.subtilis*. It should be noted that all the tRNA genes and clusters so far reported in several *Mycoplasma* and *Spiroplasma* species are not linked to rRNA operon, except that a 2-tRNA gene cluster locates at the 5'-upstream region of an rRNA operon in *M.capricolum* (8) and *Mycoplasma PG50* (30).

The arrangement of tRNA^{Ser}(GCT)-tRNA^{Glu}(TTC) in the 2-gene cluster in *A.laidlawii* can be seen at the 3'-end region of the 21-gene cluster in *B.subtilis*. The gene arrangement in the 3-gene cluster is also similar to a part of the 16-gene cluster (see Fig. 5). The genes for tRNA^{Gly}(GCC), tRNA^{Leu}(CAA) and tRNA^{Trp}(CCA), that respectively exist as a gene, seem to have resulted by translocation either from the 21- or from the 16-gene cluster, since the homologous tRNA genes exist in either one of the *B.subtilis* clusters. Altogether, the homologues of 19 out of 22 tRNA genes found in *A.laidlawii* exist in either 21- or 16-gene clusters in *B.subtilis*. Thus, most of the *A.laidlawii* tRNA genes, like those of *M.capricolum* (8), might share the same phylogenetic origin with the two large gene clusters in *B.subtilis*.

The 22 genes found in this study would represent approximately two-third of all tRNA genes in *A.laidlawii* (see Fig.1). Then the total number of tRNA genes would be less than 35, which is much smaller number than those in *B.subtilis*. The 51 tRNA genes reported in *B.subtilis* encode 31 different tRNA species, and thus the genes for many tRNA species occur in multiple.

Figure 5. Comparisons of the tRNA gene organizations between *A.laidlawii* and *B.subtilis*. The tRNA genes of *A.laidlawii*, and *B.subtilis* are boxed by bold and thin line, respectively. The species of tRNA genes are shown by one-letter code of the specifying amino acid and the anticodon. Presumed correspondence of tRNA genes between the two species is indicated by arrows.

In *M.capricolum*, all the tRNAs except tRNA^{Lys}(UUU) are encoded each by a single gene, showing that most redundant tRNA genes are discarded due to the constraint reducing the genome size during evolution. All the 22 tRNA genes found in this study encode different tRNA species, suggesting that many redundant tRNA genes might be discarded also in *A.laidlawii*.

Evolution of anticodons

Total 25 different anticodons in the *A.laidlawii* tRNAs have been elucidated by the present DNA and RNA analyses. Twenty-seven different anticodons in *B.subtilis* (21), and all the 28 anticodons of *M.capricolum* (6,7) have been known. In Table 1 are compared the tRNA anticodons so far known between the three species in the codon table.

For anticodons in family-boxes, mo⁵UNN anticodons are found in five boxes, Leu, Val, Ser, Thr and Ala in *A.laidlawii*. The mo⁵UNN anticodon pairs with codons NNU, NNA and NNG, but not with NNC (31). Thus, in these boxes, GNN anticodon is obligate to read NNC codons. In fact, GNN anticodons are found in Thr and Gly boxes. These suggests that four codons in most family-boxes in *A.laidlawii* are translated by two anticodons mo⁵UNN and GNN, except CGN(Arg) family-box, where the codons are read by anticodons ICG and CCG (not yet found). The number of isoacceptor tRNAs estimated by selective labelling method (Fig. 4) has also demonstrated that there exist at least two isoacceptors each for Ala, Gly and Thr, three each for Ser and Arg, and four for Leu, supporting that the four codons in these family-boxes are read each by at least two anticodons. This situation is essentially the same as that in *B.subtilis*, but is apparently different from that in *M.capricolum*. In *B.subtilis*, mo⁵UNN is so far known to be used in five family-boxes, Val, Pro, Thr, Ala, and Ser, and cmnm⁵U is used in Gly box (20). GNN anticodons have been found in Ser, Thr and Gly family-boxes. In addition, anticodon

CAG has been found in Leu(CUN) family-box. These show that the four codons in most family boxes in *B.subtilis* are translated mainly by anticodons GNN and *UNN (*U: modified U), and non-obligate CNN anticodons exist in some boxes. In *M.capricolum*, on the other hand, there is only one tRNA species with anticodon UNN (U unmodified) each for Ala, Gly, Leu, Pro, Ser and Val family-boxes, and GNN and CNN anticodons do not exist in any of the eight family-boxes. No CNN anticodon has been found in the *A.laidlawii* family-boxes so far, suggesting that many, if not all, non-obligate CNN anticodons for family-boxes are deleted also in *A.laidlawii*.

Thr tRNA with anticodon AGU, which is present in *M.capricolum*, has not been found in *A.laidlawii* and in *B.subtilis*.

There is only one tRNA with anticodon ICG in the *M.capricolum* Arg(CGN) family box; this anticodon can translate codons CGU, CGC and CGA, but not codon CGG. No tRNA capable of translating codon CGG has been found (7,8), showing that CGG is an unassigned (nonsense) codon in this bacterium (12). In both *A.laidlawii* and *B.subtilis*, only anticodon IGC has been found in the CGN box. However, since CGG codons are used in the protein genes in *B.subtilis*, and *A.laidlawii* contains at least 3 Arg tRNA isoacceptors, the anticodon CCG might exist in these species.

UNN anticodons for NNR-type 2-codon sets are found in Leu (UUR), Gln (CAR), Lys (AAR), Glu (GAR) and Arg (AGR) boxes, and the first nucleoside in the anticodons of Leu and Arg tRNAs is identified as cmnm⁵U, as in the case of most of the UNN anticodons in the *M.capricolum* 2-codon sets. The G in GNN anticodons in NNY-type two-codon sets, Tyr, Gln and Asn, is all unmodified in *A.laidlawii*, as in the case in *M.capricolum*, while the G in anticodon GUA in tRNA^{Tyr} is modified to queuosine (Q) in *B.subtilis* (32).

In *M.capricolum*, there are two Ile tRNA species with anticodon GAU and LAU, where L (lysidine) is a lysil-C (33).

Table 1. The tRNA anticodons in *B.subtilis* (B), *A.laidlawii* (A) and *M.capricolum* (M).

B				A				M				B				A				M																																															
Phe(UUU)				¹ GAA				GAA				GAA				Ser(UCU)				GGA				Tyr(UAU)				QUA				GUA				GUA				Cys(UGU)				GCA				GCA																			
Phe(UUC)																Ser(UCC)								Tyr(UAC)								Cys(UGC)																																			
Leu(UUA)				TAA				² UAA				² UAA				Ser(UCA)				TGA				³ UGA				UGA				-				(UAA)				-				-				-				Trp(UGA)				-				-				² UCA			
Leu(UUG)				CAA				CAA				⁶ CAA				Ser(UCG)												-				(UAG)				-				-				-				Trp(UGG)				CCA				CCA				⁶ CCA							
Leu(CUU)																Pro(CCU)												His(CAU)												Arg(CGU)																											
Leu(CUC)																Pro(CCC)												His(CAC)				GTG				GUG				GUG				Arg(CGC)				ICG				ICG				ICG											
Leu(CUA)								³ UAG				UAG				Pro(CCA)				³ UGG				UGG				Gln(CAA)												Arg(CGA)																											
Leu(CUG)				CAG												Pro(CAG)												Gln(CAG)				TTG				⁴ UUG				⁴ UUG				Arg(CGG)								-															
Ile(AUU)				GAT								GAU				Thr(ACU)				GGT				GAG				AGU				Asn(AAU)				GTT				GTT				GUU				Ser(AGU)				GCT				GCT				GCT							
Ile(AUC)																Thr(ACC)												UGU				Asn(AUC)												Ser(AGC)																							
Ile(AUA)				CAT				CAT				LAU				Thr(ACA)				³ UGU				³ UGU				UGU				Lys(AAA)				⁵ UUU				TTT				⁴ UUU				Arg(AGA)								⁴ UCU				⁴ UCU							
Het(AUG)				CAU				CAT				CAU				Thr(AUG)																Lys(AAG)								CUU				CUU				Arg(AGG)																			
Val(GUU)																Ala(GCU)																Asp(GAU)												Gly(GGU)																							
Val(GUC)																Ala(GCC)												UGC				Asp(GAC)				GTC				GUC				GUC				Gly(GGC)				GCC				GCC											
Val(GUA)				³ UAC				³ UAC				UAC				Ala(GCA)				³ UGC				³ UGC				UGC				Glu(GAA)																Gly(GGA)				⁴ UCC								UCC							
Val(GUG)																Ala(GCG)																Glu(GAG)				TTC				TTC				⁴ UUC				Gly(GGG)																			

The anticodons shown by Italic are those determined by RNA sequencing. The modified nucleosides at the anticodon first position are: 1, 2'-O-methylguanosine (Gm); 2, 5-carboxymethylaminomethyl-2'-O-methyluridine (cmnm⁵Um); 3, 5-methoxyuridine (mo⁵U); 4, 5-carboxymethylaminomethyluridine (cmnm⁵U); 5, 5-carboxymethylaminomethyl-2-thiouridine (cmnm⁵s²U); 6, 2'-O-methylcytidine (Cm); Q, queuosine; L, lysidine; *U, uridine with unidentified modification.

The tRNA(CAT) genes homologous to *M. capricolum* tRNA^{Le}(LAU) have been found in the *A. laidlawii* 11-gene cluster and in the *B. subtilis* 21-gene cluster, respectively. Although modification of the anticodon first position of tRNA(CAT) is unknown in these species, it may be modified to L (lysidine) like in *M. capricolum*, since the sequence of tRNA(CAT) genes of *A. laidlawii* and *B. subtilis* reveal a high similarity to tRNA^{Le}(LAT) of *M. capricolum* (87% and 86%, respectively), and the tRNA(CAT) genes locate at the same corresponding position in the homologous large tRNA gene clusters, the 21-gene cluster in *B. subtilis*, the 11-gene cluster in *A. laidlawii*, and the 9-gene cluster in *M. capricolum*, respectively (see Fig. 5).

As already reported, there is only one tRNA^{Trp} with anticodon CCA in *A. laidlawii*, showing that codon UGA is not a Trp codon (19), as in the case of *B. subtilis*. In *M. capricolum*, there are two tRNA^{Trp}s with anticodon CCA and UCA, and codon UGA is read as Trp by anticodon UCA (11). Among *Mollicutes*, several species in the genus *Mycoplasma* (*M. gallisepticum* (34), *M. pneumoniae* (34), *M. genitalium* (34), *M. hyorhinis* (35) and *M. arginini* (36)), in the genus *Spiroplasma* (*S. citri* (37) and *S. str. MQ-1* (38)) and in the genus *Ureaplasma* (*U. urealyticum* (39)), have been known to use UGA as a Trp codon. These suggest that this change is common in all species in the genera *Mycoplasma*, *Spiroplasma* and *Ureaplasma*, but not in *Acholeplasma*.

The comparisons of anticodons described above show that overall codon-recognition patterns of *A. laidlawii* resemble those of *B. subtilis* rather than those of *M. capricolum*, in spite of a large reduction in the number of tRNA genes in *A. laidlawii* as compared with that in *B. subtilis*. Several characteristic features in the anticodon compositions found in *M. capricolum*, such as occurrence of UNN (U unmodified) anticodons in many family-boxes, must have occurred in the lineage of *Mycoplasma*, after the separation from *Acholeplasma* during evolution of *Mollicutes*. The changes in the genetic code, codon UGA from stop to Trp, and codon CGG from Arg to nonsense, have also occurred in *Mycoplasma* lineage after the separation from *Acholeplasma* from their ancestor (19).

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