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Levels of tRNAs in bacterial cells as affected by amino acid usage in proteins

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ABSTRACT

Transfer RNAs of *Mycoplasma capricolum* were separated by two-dimensional polyacrylamide gel electrophoresis, and the relative abundance of each of the 28 known tRNA species was measured. There existed a correlation between the relative amount of isoacceptor tRNAs and the frequency in choosing synonymous codons that could be translated by the isoacceptors. Furthermore, it was observed that the total amount of tRNAs for a particular amino acid was paralleled by the composition of the amino acid in ribosomal proteins. A similar relationship was obtained from reexamination of the previous data on *Escherichia coli* tRNAs, suggesting that the amount of tRNAs for an amino acid is affected by the usage of the amino acid in proteins.

INTRODUCTION

All organisms must preserve a set of tRNAs that may be specific to their decoding system. Intracellular abundance of each tRNA species must be arrayed in a specific pattern to translate efficiently all protein genes. The relative amount of the tRNA species in the cell has been extensively analyzed in *Escherichia coli*, arousing a particular interest in a striking correlation of the relative level of isoacceptor tRNAs with the codon usage pattern (1,2,3). Synonymous codons preferentially used in highly expressed proteins are translated by the major isoacceptors. This tendency has been also observed in *Salmonella typhimurium* (4) and *Saccharomyces cerevisiae* (5), suggesting it to be a rather general rule among other organisms. Extensive survey of tRNA genes throughout *E. coli* genome found 46 tRNA species encoded by 79 genes (6). This as well as the previous work (3) indicates a good correlation between the tRNA content and its gene copy number. The major tRNA species are encoded by quadruplicated or triplicated genes, while minor ones are by a single copy of the gene. Thus, in *E. coli*, the gene dosage seems to be a primary factor to limit the tRNA abundance though the promoter efficiency, processing, and modifications obviously affect the tRNA gene expression (6).

The features diagnostic to *Mycoplasma capricolum* genomic DNA are its small size (7×10^8 daltons; 1070 kb)(7) and extremely low G+C content (25%)(8,9). Reflecting them, there exist only 29 tRNA species (10) in *M capricolum*, comprising 28 different anticodons, the smallest in number among genetic systems so far known except for mitochondria. All but one species are encoded by a single copy of the gene. Thus, most codons are translated each by a single anticodon, and isoacceptors occur for a few amino acids. In all family boxes except for threonine and arginine boxes, a single anticodon with unmodified uridine at its first position recognizes four codons by the 4-way wobbling of translation (10,11). In the threonine box (ACN; N: U, C, A or G), in addition to $tRNA^{Thr}_{UGU}$, there is another threonine tRNA species having an unprecedented anticodon, AGU, the first letter of which is also unmodified (12). In the arginine CGN box, there is only one anticodon ICG (10), the codon CGG being untranslatable (see below).

The low GC content of the genome reflects an extremely A- and U(T)-biased codon usage in the *M. capricolum* protein genes (13). More than 90% of the codons used have A or U at their third position (14). The amino acid assignment for two codons deviates from the universal genetic code: the codon UGA from stop to tryptophan (15, 16), and the codon CGG from arginine to unassigned (10, 17), in accordance with the existence of the tryptophan anticodon UCA and the absence of an anticodon CCG, respectively.

In the context of the above peculiarities in codon usage as well as in features of anticodons, it would be intriguing to know the relative levels of tRNA species of *M. capricolum*. Here we measured the relative abundance of each tRNA species in *M. capricolum* cells. The results not only showed the good correlation between the relative amount of isoacceptors and the frequency of the synonymous codon choice, but also connoted a new point of view on the pattern of tRNA abundance. Based on the measurement, together with the previous data on *E. coli* tRNAs (1,2) and amino acid composition of proteins, we suggest that over-all amino acid usage in proteins constrains the total amount of tRNAs for the particular amino acid.

MATERIALS AND METHODS

Preparation of tRNAs

Mycoplasma capricolum (American Type Culture Collection 27343(Kid.)) cells were grown at 37°C in medium made of 2.2% (w/v) PPLO broth (Difco), 0.1% (w/v) yeast extract, 0.2% (w/v)

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glucose, 20 mM Tris-HCl (pH 7.5), 0.01% (w/v) thallous acetate, 400 units penicillin G/ml, and 1% (v/v) calf serum (18). Nucleic acids were extracted by the direct phenol method from the harvested cells, and chromatographed on a DEAE-cellulose column after incubation at 37°C for 30 min. in 0.5 M Tris-HCl (pH 9.5) for deacylation of aminoacyl-tRNAs. tRNAs were eluted with 0.2 M NaCl from DEAE-cellulose and washed extensively by repeated precipitations with ethanol. The 3'-ends of tRNAs were labeled using T4 RNA ligase and [5'-^{32}P]pCp(3000 Ci/mmol) as described by Peattie (19).

Identification and measurement of tRNA species

The 3'-end labeled tRNAs were separated by 2-dimensional(2-D) gel electrophoresis by the method of Ikemura (20), with 14% (w/v) polyacrylamide gel in the first dimension and 22% (w/v) polyacrylamide gel containing 41.7% (w/v) urea in the second dimension. After autoradiography, each spot was cut out from the gel and its radioactivity was measured with a liquid scintillation counter. Each tRNA eluted from the gel was partially digested with RNase T1 which splits RNA into 3'-GMP and oligonucleotides with a terminal 3'-GMP. The resulting 3'-end labeled oligonucleotides were separated by 12% sequencing gel electrophoresis, resulting in the tRNA species-specific autoradiographic pattern. The tRNA species in each spot was identified by comparing the pattern with the catalog of those obtained from the 29 known species of *M. capricolum* (10).

RESULTS

Figure 1(a) shows a typical 2D-gel separation of post labeled small RNA molecules including tRNAs from *M. capricolum*. More than 30 spots, including non-tRNA species, were distinguished. The tRNA species of each spot was assigned to a known tRNA (10) by partial sequencing, and measured by counting radioactivity. Spot 11 consisted of two threonine isoacceptors, and spot 17 contained two species, $tRNA^{Arg}_{ICG}$ and $tRNA^{Leu}_{UAG}$. They were further separated by electrophoresis using an 8% polyacrylamide gel containing 50% (w/w) urea (data not shown) and their ratio was calculated. $tRNA^{Glu}$, $tRNA^{Ile}_{LAU}$, $tRNA^{Lys}_{CUU}$, and $tRNA^{Val}$ were found in multiple spots. This may be due to the incomplete modifications of their minor bases though it is not proved. Since spot 26 ($tRNA^{Tyr}$), 21 ($tRNA^{Glu}$), and 17 ($tRNA^{Leu}_{UAG}$) were usually smeared and diffused, which may be due to incomplete or labile modified bases, their measurements were rather variable among experiments. $tRNA^{Trp}_{CCA}$ and $tRNA^{Pro}$ could not be identified among the spots in the 2-D gel, probably due to their small amount or/and interference with some major species. In our previous works on tryptophan tRNAs, $tRNA^{Trp}_{CCA}$ was shown to be present in an amount of 10 to 20% of that of $tRNA^{Trp}_{UCA}$ (16). Altogether, 27 out of total 29 tRNA species were identified among 29 spots in the 2-D gel pattern (Figure 1b) and 28 species were measured as summarized in Table 1. This was based on 12 labeling experiments using 4 independent preparations of tRNAs. No substantial differences were found between the quantitative patterns of tRNAs from stationary and logarithmic-phase growing cells. There were also some other spots, the sequences of which did not match tRNA structures. tRNA-like sequences other than the known 29 species were not found as far as they were examined by partially sequencing, among the spots detected by this analysis.

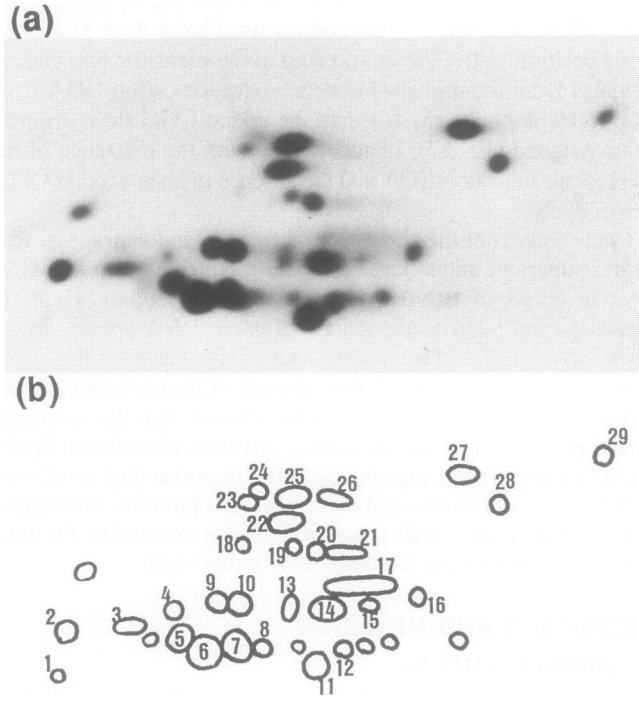

Figure 1. Separation of small RNA molecules of *M. capricolum*. (a)(3'-^{32}P)-labeled RNAs were electrophoresed in first (from left to right) and then second (from top to bottom) dimensions. (b)Schematic illustration of (a). Spots assigned to tRNA species are numbered.

Table 1. Relative amounts of tRNA species of M. capricolum

amino acid	anticodon	spot No.[1]	Quant.[2]
Ala	UGC	23	0.36
Arg	[3]ICG	17	0.21
	UCU	3	0.63
Asn	GUU	2	0.64
Asp	GUC	22	0.92
Cys	GCA	12	0.37
Gly	UCC	7	1.17
Gln	[4]UUG	4	0.17
Glu	[4]UUC	20,21	0.52
His	GUG	15	0.20
Ile	[5]LAU	13,14	0.89
	GAU		1.85
	[6]UAA	27	1.00
Leu	CAA	16	0.31
	UAG	17	0.21
Lys	[4]UUU	25	1.17
	CUU	18,19	0.24
Met	CAU	8	0.21
fMet	CAU	24	0.09
Phe	GAA	5	0.42
Pro	UGG	ND	ND
Ser	GCU	28	0.57
	UGA	29	0.35
Thr	UGU	11	0.56
	AGU	11	0.50
Trp	[6]UCA	1	0.05
	CCA	ND	0.01
Tyr	GUA	26	0.37
Val	UAC	9,10	0.85

[1]Spot number in Figure 1(a). [2]The relative amounts of the tRNAs are normalized with that of leucine $tRNA_{UAA}$ at 1.0. ND; Not measured or identified. Modification of anticodon first nucleotides: [3]inosine (I); [4]5-carboxymethylaminomethyluridine (cmnm^{5}U); [5]lysidine (L); [6]5-carboxymethylaminomethyl-2'-O-methyluridine (cmnm^{5}Um)(10).

DISCUSSION

All tRNA species of *M. capricolum* have been isolated and sequenced (10). We have also analyzed structures and organization of genes for all tRNAs in this bacterium (21). Here we measured the intracellular levels of whole tRNA species except for proline tRNA. Thus, *M. capricolum* is the first case of a genetic system whose tRNAs are characterized as a whole in their structures, genes, and quantities.

In *M. capricolum*, there are 29 tRNA species comprising 28 different anticodons, and isoacceptors occur only for arginine, leucine, lysine, serine, threonine, and tryptophan (10). In Table 2 are shown the relative contents of isoacceptor tRNAs for each amino acid and usage of synonymous codons translated by the tRNAs in the ribosomal and some other protein genes that have been sequenced (10). There can be seen a general tendency that the higher the codon usage, the higher the content of isoacceptor tRNA which translates the codons, in accordance with the results shown for *E. coli* (1), *S. typhimurium* (4), and *S. cerevisiae* (5). Minor discrepancies to this general rule exist in the leucine and serine acceptors. The amount of $tRNA^{Leu}_{CAA}$ is higher than that expected from the very low usage of the codon UUG. This is probably because $tRNA^{Leu}_{CAA}$ may have some other function than translation, such as the N-terminal addition of leucine to some proteins by the protein transferase reaction (10). For serine isoacceptors, where anticodons UGA and GCU translate 4 codons in the UCN family-box and AGY (Y: U or C) codons, respectively, $tRNA^{Ser}_{GCU}$ is higher in content than $tRNA^{Ser}_{UGA}$ in spite of the higher usage of UCN than AGY codons. The reason of this discrepancy is unknown.

We did not observe substantial differences in pattern of tRNA abundance between stationary and logarithmic-phase growing cells of *M. capricolum*. This is in contrast to a growth rate dependence of tRNA abundance in *E. coli* (22). The adaptation of intracellular tRNA level of *M. capricolum* to physiological conditions seems to be rather rigid since its tRNA system is so much simplified that isoacceptors occur for a few amino acids and they are encoded by a single copy of the gene as discussed below.

Table 2. Amount of isoacceptors and synonymous codon usages

Amino acid	Anticodon	Quant.[1]	Translatable codons	Synonymous codon usage[2]
Arg	ICG	0.21	CGU,CGC,CGA	60
	[3]UCU	0.63	AGA,AGG	192
Ile	LAU	0.89	AUA	111
	GAU	1.85	AUU,AUC	536
	[4]UAA	1.00	UUA,UUG	455
Leu	CAA	0.31	UUG	22
	UAG	0.21	CUU,CUC,CUA,CUG	99
Lys	[3]UUU	1.17	AAA,AAG	801
	[5]CUU	0.24	AAG	71
Ser	GCU	0.57	AGU,AGC	137
	UGA	0.35	UCU,UCC,UCA,UCG	282
Thr	UGU	0.56	ACU,ACC,ACA,ACG	366
	AGU	0.50	ACU	206
Trp	[4]UCA	0.05	UGA,UGG	49
	[5]CCA	0.01	UGG	7

[1]Taken from Table 1.
[2]Total number of codons translatable by the anticodon, taken from ref.9.
[3]U;5-carboxymethylaminomethyluridine (cmnm^{5}U).
[4]U;5-carboxymethylaminomethyl-2'-O-methyluridine (cmnm^{5}Um).
[5]C;(partially modified) 2'-O-methylcytidine

Table 3. Amino acid usage and relative amounts of tRNAs

Amino acid	*M.capricolum* tRNA cont.[1]	*M.capricolum* Amino acid usage[2]	*E.coli* tRNA cont.[1]	*E.coli* Amino acid usage[2]	Amino acid comp. of total protein[3]
Ala	0.36	6.6	1.00	9.6	9.6
Arg	0.84	5.2	0.92	8.0	5.5
Asn	0.64	5.4	0.60	3.2	4.5
Asp	0.92	3.6	0.80	4.2	4.5
Cys	0.37	0.4	nd	0.4	1.7
Gly	1.17	7.6	1.35	9.0	11.5
Gln	0.17	3.7	0.70	3.6	4.9
Glu	0.52	5.9	0.90	5.6	4.9
His	0.20	1.2	0.40	1.7	1.8
Ile	2.74	8.6	1.05	6.5	5.4
Leu	1.52	7.7	1.56	7.8	8.4
Lys	1.41	12.2	1.00	9.0	6.4
Met	0.30	2.3	0.30	2.8	2.9
Phe	0.42	3.5	0.35	3.2	3.5
Pro	nd	3.3	nd	3.8	4.1
Ser	0.92	5.7	0.51	4.2	4.0
Thr	1.06	6.0	0.80	5.4	4.7
Trp	0.06	0.7	0.30	0.6	1.1
Tyr	0.37	2.3	0.50	1.8	2.6
Val	0.85	7.9	1.45	9.6	7.9

[1]Total amount of tRNAs for the amino acid, normalizing that of major leucine tRNA, $tRNA_{UAA}$ in *M. capricolum* and $tRNA_{UAG}$ in *E. coli* (1), respectively, at 1.0. [2]Amino acid usage (%) in ribosomal proteins encoded by genes for S10- and spc operons in each bacterium (14). [3]Amino acid composition in total cellular proteins (%) (24). nd; Not determined.

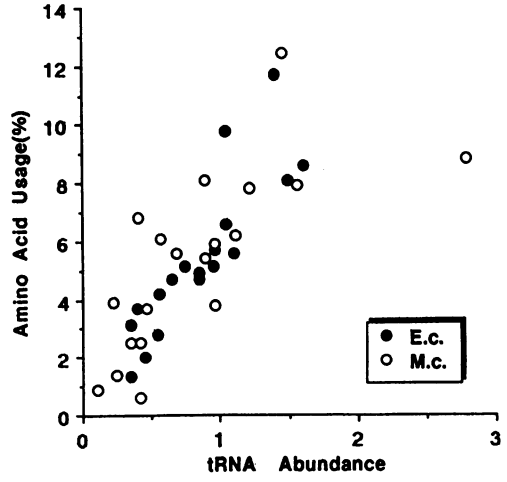

Figure 2. Co-linearity between amino acid usage and tRNA content. Amino acid compositions of ribosomal proteins of *M. capricolum* (open circles) and *E. coli* (closed circles) are plotted against the relative levels of tRNAs for the amino acid. Values in Table 3 are used.

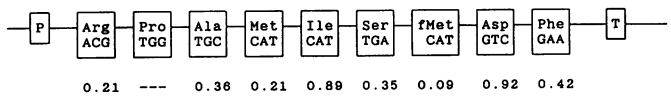

Figure 3. Gene arrangement in one of the tRNA gene operons of *M. capricolum* is schematically shown (21). The tRNA genes are indicated by boxes with specifying amino acid and anticodon sequence in DNA. Promoter (P) and terminator (T) of transcription are indicated by small boxes. On the bottom line, relative levels of tRNAs transcribed from the each gene are shown (taken from Table 1). The Content of proline tRNA has not been measured.

The relative amounts of all isoacceptors for each amino acid were summed up (Table 3) and plotted against the amino acid composition in ribosomal proteins (14) in Figure 2 (open circles). There was observed a linear correlation between them. Generally speaking, the higher the content of an amino acid in the proteins, the higher the content of the tRNA(s) that translates the codons for the amino acid. The only exception was the isoleucine tRNAs that are at an extraordinarily high level; the reason for this is unknown. A similar analysis was done using previous data for *E. coli* tRNAs (1,2) and the amino acid composition of ribosomal proteins, with results comparable with those of *M. capricolum* (Figure 2; closed circles). Since ribosomal proteins make up a large fraction of cellular proteins, their amino acid composition would represent the total usage pattern of amino acids in proteins. In fact, the usage of amino acids in ribosomal proteins in *E. coli* is almost comparable with the amino acid composition of *E. coli* total proteins (Table 3). Thus, the amount of tRNAs for each amino acid seems to be regulated to keep a particular level in parallel with the requirement of the amino acid for total protein synthesis.

Since most proteins are conservative in their amino acid sequences, the patterns of amino acid usage at least among eubacteria do not differ much. The fact that the levels of tRNAs for each amino acid are correlated with the amino acid usage implies that the pattern of tRNA levels for the amino acid is rather invariable among eubacteria. Actually, both amino acid composition and pattern of relative amount of tRNA for amino acid in *E. coli* and *M. capricolum* resembled each other on the whole (Table 3). This is in strong contrast to the codon usage patterns (choice among synonymous codons) of eubacteria, hence the pattern of relative amounts of isoacceptor tRNAs different from one species to another.

Although the amino acid composition in *M. capricolum* and *E. coli* ribosomal proteins are very similar (Table 3), there were minor differences in composition of some amino acids. Alanine, arginine, glycine, and valine showed higher scores in *E. coli* compared to those in *M. capricolum*, and conversely asparagine, isoleucine, lysine, and serine were lower. These differences in amino acid composition seemed to be dependent on the G+C content in their genomes (23). The amino acids encoded by AU-rich codons tended to be more used in *M. capricolum* (25% genomic GC content) than in *E. coli* (50%), and conversely amino acids encoded by GC-rich codons were used less (14). It is noteworthy that even though the G+C content-dependent difference in the amino acid composition is minor, tRNA levels seem to have adapted to it. Most notably, for example, lysine (AAR; mainly AAA) had higher usage in ribosomal proteins of *M. capricolum* than that of *E. coli*, and glycine (GGN) and alanine (GCN) were less (Table 3). The relative level of lysine tRNAs in *M. capricolum* is significantly higher than that in *E. coli*, and those of glycine and alanine tRNAs are lower (Table 3).

The co-linearity between amino acid usage in protein and the tRNA amount for the amino acid was stronger in *E. coli* than in *M. capricolum* (Figure 2). This may be attributed to the differences of copy number of tRNA genes between the two bacteria. Many tRNAs of *E. coli* are encoded by multiple copies of the gene, and there is a good correspondence between gene copy number and tRNA content (2,3,6). Thus, adaptation of the tRNA level to the demand of amino acid usage might be achieved grossly by gene dosage and tuned more finely by transcriptional regulation at the multiple promoters. On the contrary, however, the differences in amount of various tRNAs in the *M. capricolum* cell cannot be attributed to gene dosage effects since all tRNAs except $tRNA^{Lys}_{UUU}$ are encoded by a single gene each (21). Twenty-two out of 30 tRNA genes are arranged in five clusters consisting of 9, 5, 4, 2, and 2 genes, respectively, representing five transcriptional units (21). In Figure 3, as an example of tRNA gene organization, are shown the structure of 9 tRNA genes cluster of *M. capricolum*, together with the relative amounts of their products. Not all tRNAs transcribed from the same operon exist in the same amount in the cell, and the order of the tRNA gene arrangement in the operon does not reflect the levels of their products. These facts imply that the intracellular tRNA levels are regulated not only by promoter efficiencies for the tRNA genes but strongly by other processes such as processing of tRNA precursors and/or degradation of the products. Operation of such regulatory mechanisms seems to be adaptive so as to adjust the tRNA levels for translation. In other words, the intracellular tRNA levels are the results of a passive process that is affected by predetermined over-all codon (amino acid) usage in messenger RNAs.

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