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Codon usage revisited: Lack of correlation between codon usage and the number of tRNA genes in enterobacteria

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ABSTRACT

It is widely believed that if a high number of genes are found for any tRNA in a rapidly replicating bacteria, then the cytoplasmic levels of that tRNA will be high and an open reading frame containing a higher frequency of the complementary codon will be translated faster. This idea is based on correlations between the number of tRNA genes, tRNA concentration and the frequency of codon usage observed in a limited number of strains as well as from the fact that artificially changing the number of tRNA genes alters translation efficiency and consequently the amount of properly folded protein synthesized. tRNA gene number may greatly vary in a genome due to duplications, deletions and lateral transfer which in turn would alter the levels and functionality of many proteins. Such changes are potentially deleterious for fitness and as a result it is expected that changes in tRNA gene numbers should be accompanied by a modification of the frequency of codon usage. In contrast to this model, when comparing the number of tRNA genes and the frequency of codon usage of several *Salmonella enterica* and *Escherichia coli* strains we found that changes in the number of tRNA genes are not correlated to changes in codon usage. Furthermore, these changes are not correlated with a change in the efficiency of codon translation. These results suggest that once a genome gains or loses tRNA genes, it responds by modulating the concentrations of tRNAs rather than modifying its frequency of codon usage.

1. Introduction

Proteins, which are essential for the physiology of all organisms, are coded in nucleic acids. In order to translate the genetic message contained in nucleic acids into a polypeptide, organisms use a code where each three contiguous nucleotides (a codon) are translated to a specific amino acid. tRNAs are essential for this process. In one side of the folded tRNAs there is a loop that contains a three nucleotide sequence called the anticodon, that can interact specifically with the complementary codons in mRNAs during translation. The 3′-extreme of the tRNA is able to carry an amino acid that can be transferred to a nascent peptide. This reaction is catalyzed by the ribosome that additionally ensures a correct matching between the codon in the mRNA and the anticodon in tRNA [1–3].

Although there are some exceptions, in most organisms and growth conditions each codon codes for a single amino acid with high precision [4–7]. Nevertheless, the standard genetic code is redundant with 61 codons that code for only 20 canonical amino acids. Thus, while Met and Trp are coded by a single codon, Arg, Leu and Ser are coded by as many as 6 different codons [8]. Thanks to wobble interactions -where the ribosome allows interactions between non Watson-Crick base pairs at the third codon position- a tRNA can recognize several codons. This allows one tRNA to decode several codons (coding for the same amino acid) and also a single codon to be decoded by several tRNAs (carrying the same amino acid). There has been a long debate regarding the role of such redundancy in protein synthesis. One of the most accepted ideas is that different codons coding for the same amino acid will be translated at a different speed. Thus, highly expressed proteins will require their genes to be coded mostly by codons that are efficiently translated which are expected to correlate with high cellular levels of the corresponding tRNAs. This idea is based on the fact that translation elongation speed depends on the concentration of aa-tRNAs [8]. Also, it has been shown that in rapidly replicating

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organisms, there is a positive correlation between tRNA concentration (or the number of tRNA genes, see below) and the usage of the codons they decode in highly expressed genes [8,9]. Consistent with gene copy number effects, artificially changing the concentration of tRNAs in an organism [10] or the frequency of codons in a gene [11,12] alters gene expression and cell fitness [13]. Additionally, usage of infrequent codons that are expected to be translated at slower rates has been associated with modulation of processes coupled to translation such as protein folding or secretion [3,8,11,14]. In fact, changing codon usage in genes may induce the production of incorrectly folded proteins [3,11,14].

Bacteria can easily obtain genetic material through lateral transfer, a process that is essential for a rapid adaptation of the genome to new environments. Many of the mechanisms involved in the transfer of genetic material involve the transfer of tRNA genes. For instance, viral genomes, genomic islands and plasmids have been shown to carry one or several tRNA genes [15–18]. Internal recombination of different areas of a genome can also duplicate or produce the loss of genes coding for tRNAs [18]. Thus, the number of genes coding for tRNAs in bacterial species can change in a single generation. Concentration of tRNAs has been shown to be correlated to the number of genes coding for them, at least in bacteria such as *Escherichia coli* [19,20]. Thus, changes in the number of tRNA genes are expected to alter the cellular concentration of the corresponding tRNAs. As discussed above, this is expected to modify the speed of translation of some of the codons altering the levels and functionality of at least part of the proteome.

McDonald et al. have shown that changes in the number of tRNA genes derived from lateral transfer events correlate with codon usage of accompanying genes acquired by such events in genomes of *Escherichia coli* and *Shigella*. Potentially, these correlations allow a more efficient translation of the newly acquired genes [18]. We have found previously a similar trend while analyzing codon usage and tRNA gene content of an integrative conjugative element present in the genome of the chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans* [15,21]. Nevertheless, recent reports have shown that most tRNAs in this mobile element are expressed at very low levels [22], questioning their ability to improve gene expression. If it is common that changes in tRNA gene copy numbers produce only small changes in tRNA levels, then we would expect these have minor effects on gene expression and, consequently, on the frequency of codon usage in most genes of the genome. To test this hypothesis, we studied the relationship between the number of tRNA genes and the frequency of codon usage in genomes of enterobacteria. We have selected as models two well studied enterobacteria, *Escherichia coli* and *Salmonella enterica*. Our results indicate that changes in the number of genes coding for tRNAs have only minor effects on codon usage of both the whole genome and highly expressed genes. Correspondingly, fusions of codons translated by these tRNAs to *gfp*, have little effect on its translation efficiency. These results suggest that expression of tRNA genes acquired by lateral transfer in enterobacteria is rapidly adapted to the requirements of the host genome.

2. Materials and methods

2.1. Selection of analyzed genomes

RefSeq versions of genomes from *E. coli* and *Salmonella* were downloaded from NCBI ftp site. Only genomes annotated as being at “Chromosome” or “Complete Genome” levels were used for further analyses. In order to reduce excessive sequence

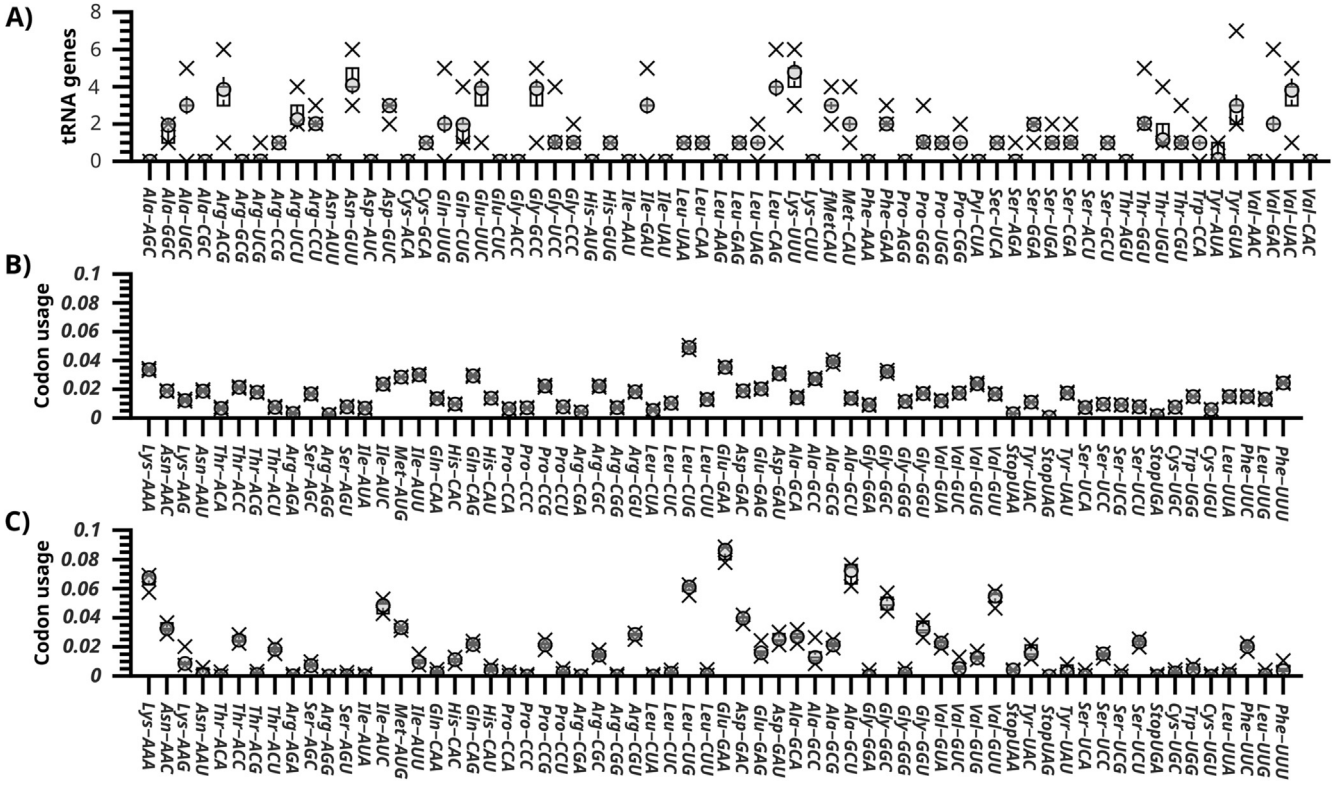

Fig. 1. tRNA gene copy numbers and frequency of codon usage in *Salmonella enterica*. The box plots show A) the average of tRNA gene copy numbers for each possible anticodon and B) the average of frequency of codon usage in all or C) a subset of highly expressed genes in 139 *Salmonella enterica* strains. In all graphs an “X” symbol indicates the most extreme values, a circle indicates the mean value and the horizontal lines of the box indicate limits where 5, 50 and 95% of the data is contained. Whiskers indicate standard deviations.

redundancy, SNP based phylogenetic trees were constructed using kSNP3 [23]. Based on these trees, a single strain was randomly selected from each group of similar strains. For example, only one genome was retained from ~30 genomes that grouped with *E. coli* K12 strains (most of which were annotated as K12 strains or strains derived from it). Using this selection method we retained 206 strains of *E. coli* and 139 strains of *S. enterica* (Supplementary file 1).

2.2. Counts of codons and tRNA genes

To prevent differences in tRNA number arising from potentially different criteria used during genome annotation, tRNA genes were re-annotated using tRNAScan-SE 2.0 [24]. Frequency of usage of each codon from each gene was calculated using in home written Perl 5 scripts. Then, average and standard deviation values were calculated from the complete set of codon usage values for each annotated gene in the genome. Additionally, a similar calculation was performed using only genes of 5 highly expressed genes selected based on a report by Karlin et al. (2001) [25]. Selected genes were *rplL* (50S ribosomal subunit protein L7/L12), *rpsA* (30S ribosomal subunit protein S1), *rpsF* (30S ribosomal subunit protein S6) *groEL* (60 kDa chaperonin) and *eno* (enolase). The *rpsF* gene was absent in one of the selected *E. coli* strains (RefSeq_assembly_accession: GCF_000013305.1), so this genome was eliminated from codon usage analyzes of highly expressed genes.

2.3. Determination of codon translation efficiency

Plasmids carrying fusions of Glu and Ala codons were constructed as indicated in supplementary methods and transformed in *S.* typhimurium ATCC strain 14028, designated 14028s, *S.* enteritidis PT4 NCTC 13349 [26] and *S.* typhi STH2370 [27]. M9 media (47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% Glycerol) supplemented with 50 μM L-Cys, 50 μM L-Trp, $MgCl_2$ and 100 μg/ml ampicillin were inoculated with each strain from a saturated overnight culture (20 h in M9 media supplemented with 0.1% tryptone and 100 μg/ml ampicilin) and grown at 37 °C in an orbital shaker. When bacteria reached mid-log phase (OD_{600} ~0.4–0.6), 50 μl aliquots were used to inoculate fresh 150 μl of media supplemented with arabinose (0.4% final concentration) in a 96-well optical-bottom plate. Plates were further shaken for 1.5 h at 37 °C. Then, OD_{600} and fluorescence intensity of GFP (Ex. 480 ± 4.5 nm, Em. 515 ± 10 nm) and mCherry (Ex. 555 ± 4.5 nm, Em. 600 ± 10 nm) were measured in a microplate reader (INFINITE M200PRO, TECAN).

3. Results

In order to compare the number of tRNA genes with the frequency of codon usage, complete sequences of genomes from *Escherichia coli* and *Salmonella enterica*, two very well characterized representatives of the enterobacteriaceae family, were obtained from NCBI and further analyzed as specified above to determine the number of tRNA codons and the frequency of usage of each codon. While the number of genes coding for tRNAs with anticodons such as Ala-UGC and Arg-UCU in *E. coli* or Ala-GGC and Arg-UCU *S. enterica* was variable between strains, others tend to be coded by a single gene copy number (eg Cys-GCA was coded by a single gene in all analyzed strains) (Fig. 1 and S1). Interestingly, the group of tRNAs that are coded by a variable number of genes is different between both species. Compared to the variability found in the number of tRNA genes, the frequency of codon usage was much more stable. This is observed for both species as well as when analyzing either the frequency of codon usage of the full genome or for a selected group of highly expressed genes (Fig. 1 and S1). The fact that the frequency of codon usage remains constant while the number of genes coding for the corresponding tRNAs varies, suggests that the correlation between the number of tRNA genes and the codon usage frequency is not as strong as usually expected.

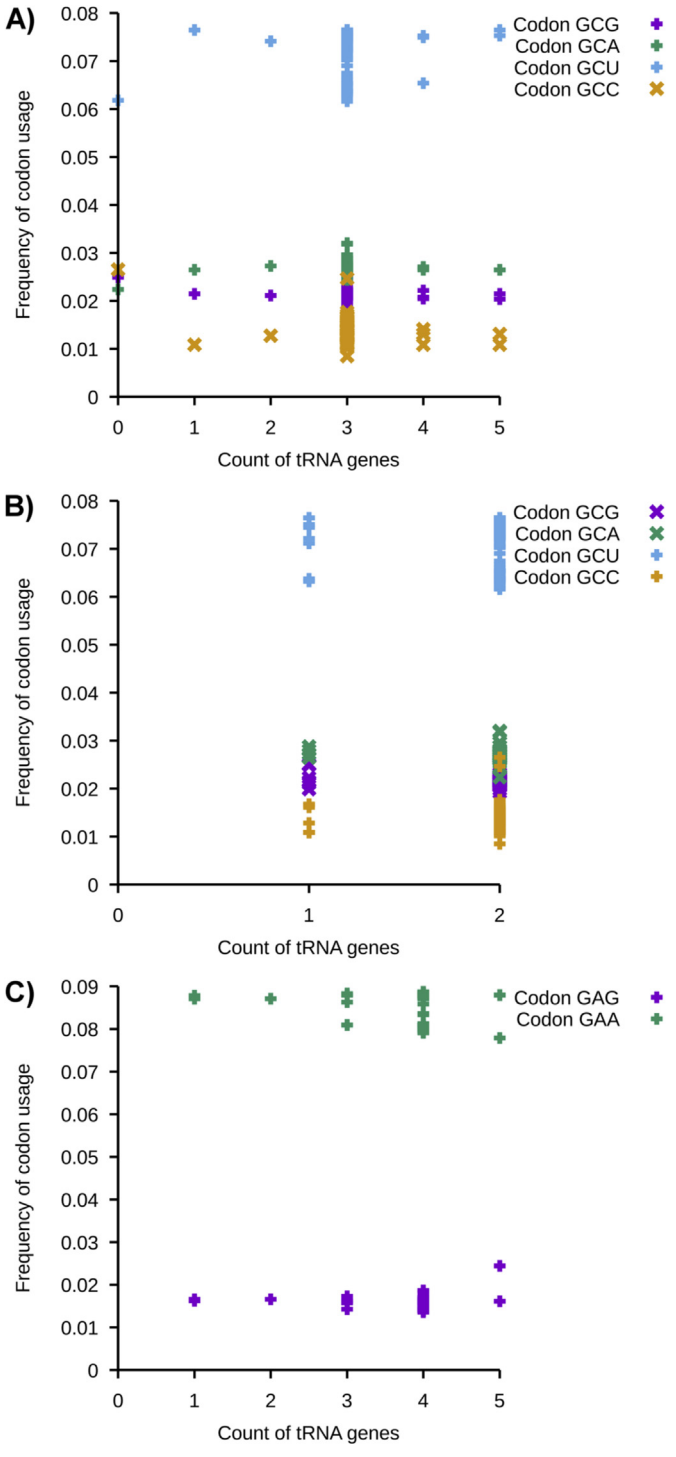

Fig. 2. Relations between frequency of codon usage in highly expressed genes and number of tRNA genes in *Salmonella enterica*. Comparison of the number of genes for A) $tRNA^{Ala}_{UGC}$, B) $tRNA^{Ala}_{GGC}$ and C) $tRNA^{Glu}_{UUC}$ with the frequency of usage of all codons decoding the corresponding amino acid in highly expressed genes of each studied *S. enterica* genome. Cognate codons are symbolized by a "+" symbol while non-cognate codons by an "X" symbol. Genes for tRNAs with other anticodons for these amino acids were not found in *S.* enterica genomes. Thus, corresponding graphs are shown in Fig. S2.

Table 1
Number of tRNA genes for Glu and Ala and the corresponding translated codons in *Salmonella enterica* strains used in translation efficiency experiments.

	$tRNA^{Glu}_{UUC}$	$tRNA^{Glu}_{CUC}$	$tRNA^{Ala}_{AGC}$	$tRNA^{Ala}_{GGC}$	$tRNA^{Ala}_{UGC}$	$tRNA^{Ala}_{CGC}$
tRNA genes in:						
S. Typhimurium 14028S	4	0	0	2	3	0
S. Typhi STH2370	1	0	0	2	1	0
S. Enteritidis PT4 NCTC13349	4	0	0	2	3	0
Codons translated[a]	GAA, GAG	NA	NA	GCC, GCU	GCA, GCG, GCU	NA

[a] NA indicates not applicable, tRNA gene not present in the corresponding genome.

3.1. Changes in the number of tRNA genes are not correlated to modifications in the frequency of codon usage

We compared the number of tRNA genes in each strain with the frequency of usage of all codons coding for the corresponding amino acid in a subset of highly expressed genes, where we expect a higher correlation between the number of tRNA genes and frequency of codon usage. A graph for each possible tRNA anticodon was produced. As observed in Fig. 2 for $tRNA^{Glu}_{UUC}$, $tRNA^{Ala}_{GGC}$ and $tRNA^{Ala}_{UGC}$ in *S. enterica* the frequency of codon usage is completely independent from the number of genes coding for the tRNAs decoding those codons or those decoding the other codons for the same amino acid. Similar results were obtained for all other tRNAs in either *S. enterica* or *E. coli* (Figs. S2 and S3). Also, similar results were obtained analyzing the full genome (Data not shown). Thus, our data indicate that when the number of tRNA genes vary in a genome there is no effect on the frequency of codon usage. This observation does not directly imply an absence of correlation between both values. In fact, when we compare the average codon usage with the average number of tRNA genes reading the corresponding codons by direct Watson-Crick complementarity, we observe a positive correlation similar to what has been previously observed (Fig. S4). Nevertheless, as mentioned previously, variability in the number of tRNA genes is much wider than observed for the frequency of codon usage.

3.2. Changes in the number of tRNA genes are not correlated to changes in the efficiency of codon translation

Changes in the number of tRNA genes are usually expected to modify the concentration of the corresponding tRNAs and as a result also alter the efficiency of codon translation. Due to the broad spectrum of effects that this would produce, it is expected that changes in the number of tRNA genes would constitute a selective pressure to change the frequency of codon usage. As this is not observed, we hypothesize there is no significant change in translation efficiency in strains with altered tRNA gene copy numbers. To test this hypothesis we constructed reporters of codon translation efficiencies by introducing 4 contiguous identical codons near the 5′ extreme of the green fluorescent protein gene (*gfp*). In addition to *gfp*, a gene coding for a red fluorescent protein (mCherry) was introduced as a transcriptional fusion to normalize for potential changes in the mRNA levels. Thus, changes in the efficiency of translation of a codon are expected to alter the ratio between fluorescence of GFP and mCherry. Reporters were constructed for codons coding for Glu (GAG and GAA) and Ala (GCG, GCA, GCT, GCC) and tested in three *S. enterica* strains that have different numbers of genes for tRNAs decoding those codons (Table 1). We observed a correlation between the GFP/mCherry ratios of Glu codons and their frequency of usage in highly expressed genes (Fig. 3A). Nevertheless, this correlation was not observed for Ala codons, a

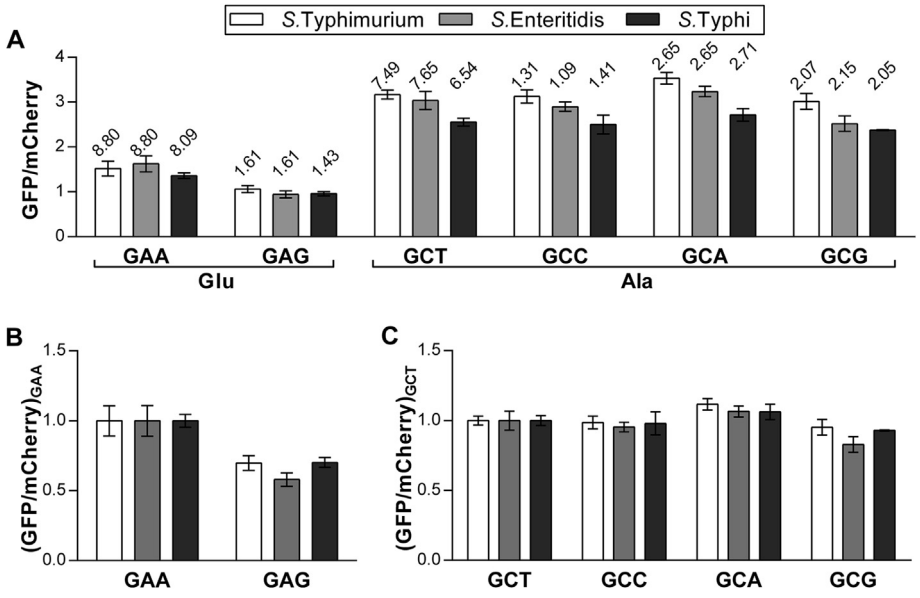

Fig. 3. Efficiency of codon translation is similar between *Salmonella enterica* strains that have different numbers of tRNA gene copies. Four contiguous identical codons were cloned in an arabinose inducible plasmid as a translational fusion to *gfp* and in transcriptional fusion to *mCherry*. The graphs show the fluorescence of GFP normalized by that of mCherry for each plasmid transformed in three different *S. enterica* serovars. A) GFP/mCherry fluorescence ratios for Glu and Ala codons. Values above the bars indicate the frequency of codon usage (in percent) in highly expressed genes of the corresponding strains. B) and C) show the same data normalized by the average GFP/mCherry fluorescence ratio of B) GAA Glu codon and C) GCT Ala codon. *S.* Typhimurium data is show in white bars, *S.* Enteritidis in light gray bars and *S.* Typhi in dark gray bars.

result in accordance with previous reports showing a similar translation efficiency for all codons coding diverse amino acids [10]. Given that the number of tRNAs that decode each Ala codon are different, this result suggests that efficiency of translation is not correlated with the number of tRNA genes. Furthermore, when the fluorescence ratios are normalized by the ratio of one of the reporters it is evident that relative efficiencies of codon translation are equivalent in the three *S. enterica* strains (Fig. 3B and C), although *S.* Typhi has a much lower number of genes coding for $tRNA^{Glu}_{UUC}$ and $tRNA^{Ala}_{UGC}$ than the other strains, supporting the idea that under exponential growth the number of tRNA genes is not affecting codon translation efficiencies.

4. Discussion

Sudden changes in cellular levels of tRNAs such as those expected by alteration in tRNA gene numbers due to lateral transfer of genes, gene duplication and deletions or mutations of anticodons should produce global changes in the concentration and folding of proteins. Nevertheless, in this work we have observed that changes in the number of tRNA genes is not correlated to alterations in the frequency of usage or the translation efficiency of the corresponding codons in enterobacteria. Thus, codon usage and translation efficiency seem to be evolutionary robust, while the tRNA gene copy number is apparently less constrained. This is in agreement with a previous report by Satapathy *et al.* who have questioned the role of tRNA gene numbers in selection of codon bias, based on the absence of correlation between the number of tRNA genes and enrichment of C vs U ending codons in the case of 4 amino acids coded by two codons translated by a single tRNA isoacceptor [28]. Probable mechanisms allowing this stability in codon usage in organisms where tRNA gene numbers vary, are silencing of acquired tRNA genes as has been observed in *A. ferrooxidans* [22] or regulation of either transcription or stability of tRNAs which has also been observed under a limited number of experimental conditions [29,30]. Both mechanisms are expected to occur much faster than changes in codons usage, which would require a much larger number of mutations [31]. The fact that genes in mobile elements are enriched for codons decoded by tRNAs contained in these elements [18,21] might be explained by either 1) codon usage of acquired protein coding genes being adapted to genomic tRNA availability slower than silencing of acquired tRNA genes, 2) retaining the original codon usage of acquired genes being advantageous to recipient cells because it reduces the expression of foreign genes that could reduce cellular fitness, or 3) both the tRNA and the protein genes only being expressed in particular conditions, thus reducing the pressure to adapt the codon usage of the rest of the genome.

In any case, based on our observations the estimation of translation efficiency using parameters based on codon usage of highly expressed genes (such as CAI [32]) seems to be more reliable than those based on the count of tRNA genes (such as TAI [33]). Still, caution should be taken when using such parameters as under several culture conditions levels of tRNAs [29,30], their aminoacylation [34], or levels of chemical modification [35] can be altered, changing the efficiency of translation [10,12,36].

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Transparency document

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Appendix A. Supplementary data

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