INCORRECT HETEROLOGOUS AMINOACYLATION OF VARIOUS YEAST tRNAS CATALYSED BY *E. COLI* VALYL-tRNA SYNTHETASE

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1. Introduction

The specificity of the aminoacylation reaction of tRNAs is necessary for the correct incorporation of amino acids into proteins. This specificity is often maintained in systems where the tRNA and the corresponding aminoacyl-tRNA synthetase are from different sources [1]. However, several cases of incorrect aminoacylation have been reported in heterologous systems involving a tRNA and an aminoacyl-tRNA synthetase which were not specific for the same amino acid [1–5]. In particular, it has been shown that it is possible to attach phenylanaline to $tRNA_I^{Val}$ (*E. coli*) [4, 5a] and to $tRNA_I^{Ala}$ (*E. coli*) [5b, 6] in the presence of phenylalanyl-tRNA synthetase from yeast. This incorrect heterologous aminoacylation can be related to the existence of some structural analogies between $tRNA_I^{Val}$ (*E. coli*) and $tRNA^{Phe}$ (yeast). However, it has not so far been possible to demonstrate the reciprocal reaction, i.e. the esterification of $tRNA^{Phe}$ (yeast) with valine by valyl-tRNA synthetase from *E. coli* ($VRS_{E.\ coli}$) [4].

It is shown in the present work that when special experimental conditions are used, different from those of Taglang et al. [4], it is possible in the presence of $VRS_{E.\ coli}$ to attach valine to $tRNA^{Phe}$ (yeast) and also to other yeast tRNAs such as $tRNA^{Ala}$ $tRNA^{Ile}$ and $tRNA^{Thr}$, in addition to the already known heterologous aminoacylation of $tRNA^{Val}$ (yeast) [7]. Our results are discussed in relation to some common structural characteristics of those tRNAs which can be aminoacylated by $VRS_{E.\ coli}$.

2. Materials and methods

2.1. *Aminoacyl-tRNA synthetases*

$VRS_{E.\ coli}$ (21 units/mg) was prepared according to Yaniv and Gros [8]. VRS_{yeast} (5 units/mg) was purified according to a simplified procedure [6] derived from the original method of Lagerkvist and Waldenström [9]. One unit of enzyme catalyses the esterification of 1 μmole valine to tRNA after 10 min under standard conditions [8, 10].

A crude extract from baker's yeast was used as a total aminoacyl-tRNA synthetase preparation [11].

2.2. *tRNAs*

$tRNA_I^{Val}$ (*E. coli*) was purified from total *E. coli* tRNA (Schwarz, Orangeburg, USA). $tRNA_I^{Val}$ was separated from $tRNA_{II}^{Val}$ on a BD-cellulose (20–50 mesh, Schwarz) column [12] and purification was achieved according to Gillam et al. [13]. One sample was a kind gift from Dr. Kelmers (Oak Ridge National Laboratory, USA).

* with the technical assistance of M. Delfau.

Table 1
Aminoacylation of total yeast tRNA by VRS_{yeast} or by $VRS_{E.\ coli}$.

	Classical aminoacylation conditions		Special aminoacylation conditions	
	Without	With 20% DMSO	Without	With 20% DMSO
VRS_{Yeast}	1.85 (a)	2.00 (a)	1.88 (c)	1.80 (c)
$VRS_{E.\ coli}$	1.75 (b)	1.95 (b)	2.65 (c)	6.10 (c)

Experimental conditions: Tests are performed in a volume of 100 µl containing 50 µg total yeast tRNA. (a) [10] and (b) [8] are conditions usually used for classical aminoacylation of tRNAs by their cognate aminoacyl-tRNA synthetases. We use per test 5 mU VRS_{yeast} or 20 mU $VRS_{E.\ coli}$. (c) special aminoacylation conditions. Each sample contains: 0.05 µmole ATP; 0.75 µmole $MgCl_2$ ($[Mg^{2+}]/[ATP] = 15$); 10 nmoles ^{14}C-valine (20µCi/µmole); 5 µg bovine serum albumin and variable amounts of enzyme (5 to 25 m units VRS_{yeast} or 100 m units $VRS_{E.\ coli}$). In some cases 20% DMSO is added. Samples are incubated 2 hr at 30°. The radioactivity of valyl-tRNAs is measured following the method of Mans and Novelli [18]. Results are expressed in nmoles ^{14}C-valine per 1 mg tRNA (1 mg tRNA corresponds to 37 nmoles).

Yeast tRNAs were purified from total brewer's yeast tRNA (Boehringer, Mannheim, Germany). $tRNA^{Phe}$ was obtained pure after tRNA fractionation by countercurrent distribution [14]. The other yeast tRNAs were obtained from partially purified countercurrent fractions. Purifications were completed by chromatography. $tRNA^{Ala}$ was obtained pure after two chromatographic separations on BD-cellulose (20–50 mesh) columns at pH 6.8 and pH 4.0. The tRNA was eluted with NaCl gradients containing 0.01 M $MgSO_4$. $tRNA_{II}^{Val}$ and $tRNA^{Asp}$ were prepared respectively in our laboratory by J. Bonnet [15] and by G. Keith [16]. It was verified that $tRNA^{Phe}$, $tRNA^{Ala}$ and $tRNA^{Asp}$ were absolutely free from any contamination by $tRNA^{Val}$.

Periodate oxidation of tRNAs was performed according to ARCA et al. [17].

2.3. *Aminoacylation conditions*

Aminoacylation was performed using either the classical conditions described for the *E. coli* system [8] and for the yeast [10], or special conditions (see legend of table 1) very similar to those used by Ritter et al. [3] for the abnormal aminoacylation of $tRNA^{Val}$ (*E. coli*) by *Neurospora crassa* phenylalanyl-tRNA synthetase. These special conditions differ from the classical ones by a low ionic strength (4×10^{-2} M instead of 12×10^{-2} M), the absence of monovalent cations such as Na^+ or NH_4^+, a $[Mg^{2+}]/[ATP]$ ratio of 15 instead of 1.5, pH 8.5 instead of pH 7.5, high enzyme concentrations, a long reaction time (2 hr instead of 30 min) and in some cases the addition of an organic solvent (dimethylsulfoxide = DMSO).

In this work the reaction rates were not determined, but the levels of the charge of the tRNAs were measured.

3. Results

Table 1 shows that, using the classical aminoacylation conditions, the same amount of valine can be attached to total yeast tRNA in the presence of VRS either from yeast or from *E. coli*. No additional aminoacylation is observed in the presence of DMSO. This suggests that in these conditions there is no aminoacylation of tRNAs other than $tRNA^{Val}$.

When the special aminoacylation conditions are used, the same level of aminoacylation of yeast tRNA as in the preceeding experiment is observed when VRS yeast is used, suggesting that no errors occurred in this homologous system. However, using the same special conditions in the presence of $VRS_{E.\ coli}$, there is an additional aminoacylation of yeast tRNA which is especially high in the presence of 20% DMSO, suggesting that tRNAs other than $tRNA^{Val}$ have been aminoacylated.

In order to characterize these other yeast tRNAs

Table 2
Aminoacylation of periodate oxidized [17] and then deacylated [19] total valyl-tRNA (yeast).

Amino acids	– DMSO		+ DMSO	
	a	b	a	b
Valine	1.49	83	1.47	82
Threonine	0.09	11	0.28	33
Isoleucine	0.33	15	0.59	27
Phenylalanine	0.16	10	0.38	23
Alanine	0.04	3	0.31	23
Methionine	0.07	12	0.10	17
Proline	0.04	8	0.05	10
Histidine	0.03	5	0.05	9
Tryptophan	0.00	0	0.10	7
Tyrosine	0.09	8	0.05	5
Leucine	0.02	2	0.10	4
Glycine	0.01	0	0.08	4
Aspartic acid	0.02	1	0.05	3
Glutamine	0.00	0	0.04	2
Lysine	0.00	0	0.03	2
Arginine	0.01	0	0.03	1
Glutamic acid	0.01	1	0.02	1
Serine	0.04	1	0.04	1

Total yeast tRNA was aminoacylated with valine using the special conditions in the presence of $VRS_{E.\ coli}$ (125 mU per 100 μg tRNA and per 100 μl incubation mixture) with or without DMSO. For these experiments two blanks were performed: one without tRNA and the other one with periodate oxidized but non aminoacylated tRNA. Chemical aminoacylation conditions for the yeast system and a crude enzyme preparation from bakers yeast were used [11]. (a) nmoles aminoacid aminoacylated per 1 mg tRNA; (b) percentage aminoacylation of treated yeast tRNA to non treated yeast tRNA. Experimental values are mean values of three experiments, each of them being performed twice.

which attach valine, the total yeast tRNA was aminoacylated by $VRS_{E.\ coli}$ in the special conditions described and then oxidized with periodate. After this treatment which oxidizes only the non-aminoacylated tRNAs, the valine was removed from the protected tRNAs and the amino acid accepting capacity of these undegraded tRNAs was determined in the presence of a crude yeast enzyme extract. In this way it was possible to characterize tRNAs loaded with valine. It was verified that in these experiments the amount of aminoacylation was not significantly reduced by a possible competitive inhibition by periodate oxidized yeast tRNAs. Table 2 shows that several yeast tRNAs other than $tRNA^{Val}$, e.g. $tRNA^{Phe}$, $tRNA^{Ala}$, $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Met}$, can be significantly aminoacylated with valine by $VRS_{E.coli}$. Several errors perhaps occur with other tRNAs, i.e. $tRNA^{Pro}$, $tRNA^{His}$ and $tRNA^{Try}$, but further experiments must be performed with the corresponding isolated tRNAs in order to check whether these results are significant. It must be emphasized that in all cases where these errors seem to occur, the addition of DMSO increases their level.

It was possible to confirm some results of table 2, using purified yeast tRNA (5 μg tRNA per test) instead of the total yeast tRNA. An incorrect heterologous aminoacylation catalysed by $VRS_{E.\ coli}$ was observed with purified $tRNA^{Phe}$ (yeast) and $tRNA^{Ala}$ (yeast). These incorrect aminoacylations become significant only with high enzyme concentrations (75 mU of $VRS_{E.\ coli}$ per test) and in the presence of 20% DMSO. Using these conditions, high percentages such as 50% $tRNA^{Phe}$ (yeast) and $tRNA^{Ala}$ (yeast) can be charged with valine. Without the organic solvent, a limited aminoacylation still occurs for $tRNA^{Phe}$ (yeast), whereas no loading takes place for $tRNA^{Ala}$ (yeast). $tRNA_I^{Val}$ (*E. coli*) and $tRNA_{II}^{Val}$ (yeast) are already completely aminoacylated in the presence of low enzyme concentrations (0.1 mU $VRS_{E.\ coli}$ per test) and without DMSO. In the case of $tRNA^{Asp}$ (yeast), which gave a negative result in table 2, no aminoacylation can be observed in any experimental conditions.

We have verified that these incorrect heterologous aminoacylations observed with $tRNA^{Phe}$ (yeast) and $tRNA^{Ala}$ (yeast) do not correspond to artefacts. On the one hand, esterification with valine of periodate oxidized $tRNA^{Phe}$ and $tRNA^{Ala}$ was not possible, and on the other hand we could isolate a [^{14}C]-valyl-$tRNA^{Phe}$ (yeast) and a ^{14}C-valyl-$tRNA^{Ala}$ (yeast) on DEAE-cellulose columns (urea 7 M, pH 3.0). These experiments show that the incorrect aminoacylations described are not due to non specific adsorptions of radioactive valine onto $tRNA^{Phe}$ (yeast) and $tRNA^{Ala}$ (yeast).

It must be emphasized that these incorrect heterologous aminoacylations can only be observed under the special experimental conditions described in this work. A modification of one parameter of the reaction, for instance the ratio $[Mg^{2+}]/[ATP]$, can be sufficient to considerably decrease the level of the incorrect aminoacylation.

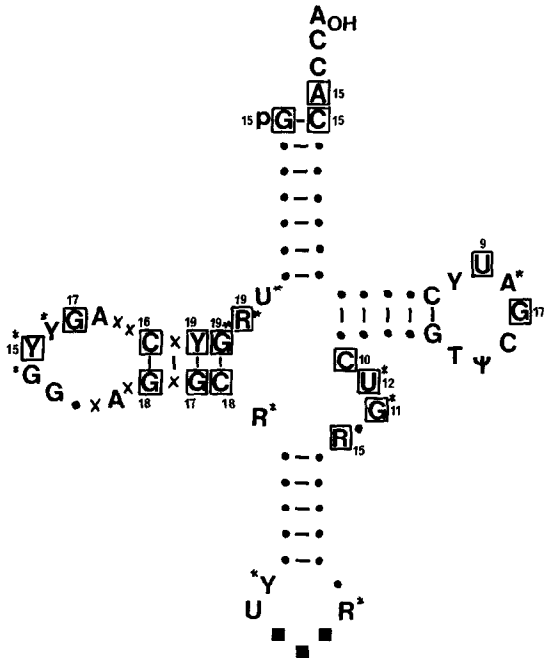

Fig. 1. Common structural features in $tRNA_I^{Val}$ (*E. coli*) [20], $tRNA_{II}^{Val}$ (brewer's yeast) [21, 22], $tRNA^{Phe}$ (yeast) [23] and $tRNA^{Ala}$ (yeast) [24, 25]. N = common nucleosides in all tRNAs of known sequence (20 tRNAs); [N] = common nucleosides in the 4 studied tRNAs. (The number written behind each of these common nucleosides indicates the number of tRNAs of known sequence containing this nucleoside) N* = modified nucleosides in some tRNA species; ■ = anticodon nucleosides; R = purine; Y = pyrimidine (replacement of a purine by another one or of a pyrimidine by another one are only mentioned when occurring in a single stranded region or in the case of G–U pair in place of a G–C pair); ● = differences; X = missing nucleosides in some tRNA species.

4. Discussion

We report in this paper that incorrect aminoacylation can be produced on heterologous yeast tRNAs by $VRS_{E.\ coli}$ under special experimental conditions. It must be pointed out that these errors are only found with a limited number of yeast tRNAs. It is likely that they are the consequence of conformational changes due to the special experimental conditions used, either of these tRNAs or of the $VRS_{E.\ coli}$ or of both. Whatever the molecular mechanism of these incorrect recognitions, some structural analogies must certainly be involved between the $tRNA_I^{Val}$ (*E. coli*) which is normally recognized by $VRS_{E.coli}$ and those yeast tRNAs which are abnormally recognized by this enzyme. These structural analogies can occur either at the level of the nucleotide sequence or the tertiary structure of these tRNAs.

Fig. 1 shows some similarities in the sequences of $tRNA_I^{Val}$ (*E. coli*) and of three tRNAs from yeast which are able to be loaded with valine under our special experimental conditions and whose primary structure is known ($tRNA^{Val}$, $tRNA^{Phe}$ and $tRNA^{Ala}$). If we take into account only common nucleotides which are not present in all tRNAs of known sequence, the most striking common features in the four tRNAs studied are located in the dihydrouracil stem (but these analogies are found in almost all tRNAs of known sequence), in the terminal part of the amino acid accepting region and especially in the extra loop which is built up with five nucleotides, three of them being common if we neglect some minor modifications.

In their work on heterologous aminoacylation of tRNAs by yeast phenylalanyl-tRNA synthetase, Dudock et al. [5b] also observed striking similitudes in the region adjacent to the dihydrouridine loop and postulated that this is the recognition site for this enzyme. Further experiments will be necessary in order to determine if the structural analogies shown in fig. 1 of our work with $VRS_{E.\ coli}$ are fortuitous or if they have a functional significance. If the latter is true, we could predict that the yeast tRNAs listed in table 2 which are aminoacylated by $VRS_{E.\ coli}$ ($tRNA^{Thr}$ and $tRNA^{Met}$ for instance) should contain the same structural features. It is interesting to note that $tRNA^{Asp}$ (yeast) [26] and $tRNA^{Ser}$ (yeast) [27] which cannot be loaded with valine do not contain the structural analogies shown in fig. 1. For instance, the extra loop has a different size and a different nucleotide sequence; the region near the amino acid accepting end is also different.

It must be emphasized that among this group of tRNAs which are recognized by $VRS_{E.\ coli}$, the incorrect aminoacylation does not take place to the same extent. These differences in reactivity could be ascribed to the regions of the tRNAs which are not identical.

These analogies or differences should not be

necessarily considered only from the point of view of the primary structure. They could be involved in the tertiary conformation necessary for enzyme recognition.

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